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THE EFFECTS OF FOUR SPECIES OF LARVAL TREMATODES UPON THE LIVER AND OVOTESTIS OF THE SNAIL, *STAGNICOLA EMARGINATA ANGULATA* (SOWERBY)

IVAN PRATT AND G. D. BARTON

Department of Zoology, University of Idaho, Moscow

A snail may shed large numbers of cercariae when confined in the laboratory for study, and yet show no signs of ill-health as a result of the presence of large numbers of parasites within its body. Brackett* (1940) stated that lack of nourishment on the part of the snail decreased the production of cercariae. When the snail was fed, it again shed cercariae.

Faust (1917) devoted some attention to the effect of larval trematodes upon several species of snails. Cytological evidence was presented to show that liver cell changes took place following invasion of the snail by the parasites. He stated, "In an examination of living material and sections of infected mollusk liver tissue, no infection was found to be so light that the host was unharmed."

Wesenberg-Lund (1934) described the effect of larval trematodes upon four species of snails. He recorded one "tumor-like" growth in a snail which he attributed to the effect of the larval parasites and stated that "The liver was almost destroyed."

Rothschild (1938) concluded that snails of the genus and species *Peringia ulvae* when parasitized by trematodes were smaller than other individuals of the same age that were not parasitized.

The present study was limited to the effect of four species of larval trematodes upon the liver and ovotestis of *Stagnicola emarginata angulata* in an effort to determine: (1) whether different species of parasites had appreciably different effects upon the same species of snail host; (2) what effect the presence of large numbers of parasites had upon the ovotestis and the tubules of the liver; and (3) whether or not parasites were found within the lumina of the liver tubules or ovotestis.

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MATERIALS AND METHODS

All the snails used in this work were *Stagnicola emarginata angulata* and were collected from Douglas Lake, Michigan. The cercariae from each snail were identified while alive and the snail was then extracted from the shell. The upper half of the snail, constituting liver to the unaided eye, was detached from the rest of the animal and the two pieces fixed together in Gilson's fluid. The specimen infected with *Plagiorchis muris* was fixed in Goldschmidt's fixative.

After fixation the specimens were transferred to dioxan until the time when they could be embedded in paraffin. The paraffin blocks were sectioned 8 to 14 microns thick, affixed to slides and stained in Semicon's carmine. When a strong, fresh stain was used, one hour in the stain was adequate to produce a sharp differentiation of the tissues. After staining, the slides were washed in 70 per cent alcohol, dehydrated in dioxan and mounted in damar.

The cercariae coming from the infected snails were identified respectively as: *Cercaria laruei* (Cort and Brooks, 1928), *Plagiorchis muris* (McMullen, 1937), *Cercaria yogena* (Cort and Brackett, 1937) and *Diplostomum flexicaudum* (Cort and Brackett, 1937). All snails studied had been shedding only one kind of cercariae at the time that they were killed. A snail which was not shedding cercariae into the water at the time of examination and in which no cercariae were found after sectioning was studied for comparisons with the infected snails. Careful examination of the entire liver of each snail was made.

DATA

The liver of an uninfected snail identified as *Stagnicola emarginata angulata* was made up of a fairly compact mass of tubules held in place by a loose network of connective tissue, the latter of which was not completely continuous. The whole mass of the liver was confined by a distinct and continuous limiting membrane. Toward the anterior end of the liver larger thin-walled ducts with a villi-like mucosa and rather large lumina connected the liver to the intestine. These have been designated liver ducts. The tubules of the liver itself were two cells thick with a distinct lumen in each tubule. An amorphous material that stained lightly with Semicon's carmine often was found in the lumen of the liver tubules. Light staining areas in the cells making up the inner wall of the tubules indicated that some soluble substance had been present which the dioxan or alcohol had removed.

In the uninfected snails the liver tubules were distributed evenly within the liver. In a cross-section in the upper third of the liver there were found to be 112 disconnected liver tubules. This number was not an average; it was recorded because it was the count on a section similar to ones studied in infected snails later in this paper.

The ovotestis of the uninfected snail was embedded within the body of the liver and was made up of a very thin-walled sac which extended a considerable distance within the liver. Within the ovotestis numerous partially developed eggs were to be seen attached to the wall. There were no sperm present, but that was explained by the fact that the snails were killed in August after the male phase had been completed and the animals were functional females.

A snail infected with *Cercaria yogena* was prepared for detailed study. One parasite was found in the small upper end of the liver and three more somewhat farther along lying just under the limiting membrane. In neither of these sites were the parasites in close contact with the liver tubules. Farther forward six specimens were found lying loosely about the hermaphroditic duct in the connective tissue which was rather abundant in that area.

At the place where the intestine reached its most posterior extent and bent upon itself toward the anterior, there were nine parasites lying alongside the intestine. Slightly anterior to this point the hermaphroditic duct came to lie between the two loops of the intestine, and the three tubes were loosely held together by connective tissue. From this point forward the parasites became more numerous and were located in this connective tissue. At the smaller anterior or lower end of the liver a count was made of liver tubules and parasites in cross-section. The section selected contained 29 liver tubules and 58 parasites.

This snail had a medium to light infection, and the parasites remained in the central area of connective tissue previously mentioned. None was found far enough toward the periphery of the liver to be surrounded completely by liver tubules with the possible exception of the two groups on the extreme upper end of the liver. The parasites did not lie against the liver tubules and none was within the lumen of either a liver tubule or a reproductive organ.

Only ova were present within the ovotestis, but the snail had been killed in August, as had the previous one. Neither the liver nor the ovotestis showed obvious signs of injury in this snail.

A snail infected with *Cercaria laruei* was carefully examined for the characteristics of that infection. The snail chosen was heavily infected so that an estimate of the number of parasites present was not attempted. At the tip of the liver beyond the ovotestis where no tubes or open spaces provided a natural avenue for penetration, four parasites were present among the tubules. A short distance toward the anterior the parasites became numerous and were concentrated in the central area of the liver, while the liver tubules were found to occupy the periphery of that organ.

Thin-walled sacs were present in the center of the liver and could be identified by location and nature of the sac wall as ovotestis, but neither

eggs nor sperm were present. A few cells were present in the sac. Similar cells had been identified as primordial germ cells in other material. The snail appeared to be sterile. No parasites were found within the lumina of the ovotestis, the various ducts or liver tubules.

A complete cross-section contained 76 parasites and 49 liver tubules. The parasites were rather small and the liver tubules were smaller than in either the negative or in the snail infected with *Cercaria yogena*. The network of connective tissue of the snail was evident only in the peripheral areas of the liver between the tubules where there were no parasites. It seemed probable that the presence of the parasites and their migrations had destroyed the central area of connective tissue which was present in the uninfected snail liver.

The snail that was infected with *Plagiorchis muris* larvae was heavily parasitized. Parasites were found among the liver tubules even far up at the tip of the liver. The tendency observed in the infection by *Cercaria yogena,* for the sporocysts and rediae to stay in the open spaces or along the outside of the loops of the intestine was not discernible in this infection. The large number of parasites present might have influenced the more general distribution among the liver tubules. The parasites did not crowd against the liver tubules, however, but shared the available space within the liver in a fashion similar to the distribution pattern of the liver tubules. The site of heaviest infection was an area that adjoined the intestine. This suggested that the path of ingress had been along the intestine.

Careful search was made for the ovotestis and related ducts, but no unmistakable part of that gland was found. The parasites were very numerous in that part of the liver where the ovotestis was imbedded in other specimens. This snail was killed and fixed at the same time of year as the other specimens in which an ovotestis was conspicuous.

A survey of the entire cross-section in the middle of the liver was made. There were 149 liver tubules and 117 parasites present. In one area where a liver tubule was flanked closely on either side by a parasite, the one end of the tubule was open, and the external cell layer of the liver tubule was absent. This was an exception and was the only one seen in thousands of liver tubules studied.

The parasites in this snail were of various sizes with numerous large cyst forms. The invasion among the liver tubules was more pronounced than in the other snails studied and the absence of an ovotestis was remarkable.

The infection with *Diplostomum flexicaudum* was heavy. An entire cross-section contained 48 liver tubules and 101 distinct parasites in addition to material not countable. The last was probably parts of cercariae already freed from their mother cyst. The parasites were crowded in amongst the liver tubules, but were thickest in the central area of

the liver. Many of the liver tubules were normal in appearance, but those in the central area of the organ where the parasites were thickest were smaller than the rest and took the stain more deeply than did the larger tubules at the periphery. Where parasites actually touched liver tubules in this snail, the outer layer of cells of the tubule did not appear modified by the proximity of the cyst.

The ovotestis was identified in this snail, but no eggs or sperm were present. The thin-walled sac was not invaded by flukes and the lumen was of normal size. Although the organ contained no eggs, there were present peculiar brown structures of irregular shape and a few cells that appeared to be primordia of germ cells.

DISCUSSION

The avenues of invasion of the snail by the larval trematodes, as far as could be determined, followed up the intestine and hermaphroditic duct and from thence into the body of the liver. The majority of the parasites remained in the central area of the liver. This was observed in the infections produced by four different species of trematodes. The parasites were found in the loose connective tissue between liver tubules and surrounding the intestine, ovotestis and hermaphroditic duct, but not within any of these ducts.

Variations in the different infections might well have been the result of differences in severity of infection. The *Cercaria yogena* infection did not seem to have harmed the snail as greatly as did the other three, but the number of parasites in the snail in this infection was markedly fewer. This infection was studied at greater length than the others because it was light enough to suggest which areas in the snail were first sought by invading trematodes.

The effect on the liver tubules was hard to demonstrate. A glance at the liver of a heavily infected snail gave the impression that the parasites had taken over the central area of the liver, and that the number of liver tubules was fewer as compared with an uninfected snail. These impressions were not quantitatively verified. The liver tubules in the center of the liver in heavily infected specimens were smaller than those on the same section at the periphery. In one case (*Plagiorchis muris*) there was no other obvious reason for the breakdown of the liver tubule in the center of a section than the close proximity on both sides of large parasites. The difference in staining of the tubules that were surrounded by parasites was probably an indication of changes or even degeneration of the liver tubule.

The ovotestis in both the uninfected snail and in the one lightly infected with *Cercaria yogena* appeared capable of producing ova. There was no evidence of any partially developed eggs in the snails infected with *Cercaria laruei* and *Diplostomum flexicaudum,* although the thin-walled

ovotestis was present and intact. The snail harboring the infection of *Plagiorchis muris* was unique in that there was no trace of the ovotestis. It seemed probable that heavy infections of trematode larvae had rendered the latter three snails sterile.

In none of the snails studied was there any evidence that the parasites penetrated into the lumen of any of the various ducts present.

SUMMARY

1. Serial sections were made of the livers of five specimens of the freshwater snail *Stagnicola emarginata angulata* (Sowerby).

2. One snail was not shedding cercariae at the time that it was killed, and microscopic examination of the whole animal showed that no parasites were present.

3. One snail was infected rather lightly with *Cercaria yogena*.

4. Three snails were heavily parasitized by *Cercaria laruei*, *Plagiorchis muris* and *Diplostomum flexicaudum* respectively.

5. No appreciable difference in method or effect of invasion by the four species of trematodes in the same species of snail could be distinguished.

6. The effects of a heavy infection was to reduce the apparent number of liver tubules and to limit the liver tubules to the periphery of the liver. The parasites filled the central area. In only one case did there seem to be evidence of destruction of liver tissue by the proximity of parasites.

7. In the snail infected with *Cercaria yogena* the ovotestis was apparently normal as compared with that of the uninfected snail. The ovotestis was present but contained no developing eggs in the two snails infected with *Cercaria laruei* and *Diplostomum flexicaudum*. The ovotestis was not present in the snail infected with *Plagiorchis muris*.

8. In no instance was a parasite found within the lumen of liver tubule, ovotestis, hermaphroditic duct, or intestine.

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THE REDISCOVERY TOGETHER WITH THE MORPHOLOGY OF THE LEECH, *BRANCHELLION RAVENELII* (GIRARD, 1850)

MARVIN C. MEYER
Department of Zoology, University of Kentucky, Lexington

Through the courtesy of Dr. Ross F. Nigrelli, of the New York Aquarium, I have been able to examine sixty-six specimens of *Branchellion ravenelii* (Girard, 1850), collected March 3, 1939, at Lemon Bay, Florida. Thirty-two of these specimens were taken from the ventral external surface of *Dasyatis hastatus* (DeKay, 1842),[1] the remaining twenty-four, from the same location on *Amphotistius sabinus* (LeSueur, 1824).

Dr. S. C. Ball, Curator, Peabody Museum of Natural History of Yale University, kindly sent me for my examination all of Verrill's leech collection which the former was able to assemble.

Branchellion ravenelii is a leech, PISCICOLIDAE, first described by Girard, 1850, from a skate taken at Charleston, South Carolina. Verrill (1874: 624) reported the species from "Vineyard Sound, on a stingray (*Myliobatis freminvellei*) [=*Aetobatus freminvillii*], in several instances, a number usually occurred together."

In describing the species Girard mistook the anterior for the posterior end and was unaware that the animal under consideration was a leech. His nebulous description, in part, follows:

> In whatever light we may view the animal kingdom, whether forming a series, one uninterrupted, from simple to more complicated forms, or composed of groups independent from each other, it remains as a fact, that the animal now under consideration, combines in itself, the general character of two distinct groups. Its general form is framed upon the plan of *Piscicola,* but, in addition, it has lateral appendages, (gills) reminding us of the same organs among annelids proper.
>
> The body is flat, elongated, terminated at both extremities by a disk, on which the animal crawls about, in the fashion of the earth measurer, caterpillar, and leeches.
>
> Indeed the body seems more alike that of a leech, thus showing a third group to which it bears a great analogy.
>
> At the conclusion of this paper, Professor Agassiz stated, that it is very rare to find intermediate animals existing between types. Now this specimen exhibits characters of three groups, and thus plainly shows that these characters can not have much value as the bases of types.

Although Diesing (1859: 482–483) listed the species (*Branchiobdella ravenelii*) in "Systema Helminthum," he did nothing more than copy Girard's confused description. Pratt included the species in his "Manual of the Common Invertebrate Animals." The foregoing are the only

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[1] The fish nomenclature here followed is that adopted by Jordan et al, 1930.

records of *B. ravenelii*. No further data regarding the species are available except in the form of the references of Diesing, Verrill, and Pratt, none of which deal with internal morphology.

Upon an examination, however, of Verrill's material, in which there was one specimen clearly belonging to the genus *Branchellion* and labeled "*Phyllobranchus ravenelii*, Vineyard Sd.," it appears questionable that he collected *B. ravenelii*. Instead, Verrill's specimen should have been assigned to the well established type species, *Branchellion torpedinis* Savigny, 1820, since it possesses thirty-three pairs of lateral, broad, foliaceous, lobed or scalloped, branchiae. If the figure accompanying Verrill's article (Pl. XVIII, Fig. 89[2]) is based on the specimen examined, he erred, because in the drawing only thirty-two pairs of such branchiae are shown. Because only one specimen was available, it was not possible to dissect or section it in order that a comparison between the reproductive systems of *B. ravenelii* and *B. torpedinis*, as worked out by Sukatschoff, could be made.

The most striking characteristic of *B. ravenelii* consists in its having thirty-one pairs of lamellar branchiae along each side of the posterior $\frac{3}{4}$ of the body. It is also to be noted that the posterior sucker (anterior of Girard and of Diesing) possesses about 400 cupules situated on its ventral surface. The body is sharply demarcated into trachelosomal, pre-urosomal, and urosomal regions and the sucker belonging with the first and third. The two anterior rings of the urosome extend forward over the smaller anterior trachelosome, forming the pre-urosome or "collar" of some authors.

For study the material was prepared in the same manner as described in a previous publication by the writer (1940: 335). The species under consideration consequently corresponds to the synonyms and the following diagnosis:

(Figs. 1–3)

Synonyms: *Branchellion revenelii* (Girard, 1850); *Phyllobranchus ravenelii* Girard, 1850; *Branchiobdella ravenelii* Diesing, 1859; *Branchellion reveneli* Pratt, 1916.

Body is sharply divided into two regions; the trachelosomal comprises about $\frac{1}{4}$ the total length and is composed of twelve segments, the urosomal consists of twenty-two somites and comprises the remaining $\frac{3}{4}$ the total length. Ratio of over-all length to width about 10: 1. Somites in the region of the testes composed of three distinct annuli. The anterior sucker is small as compared with the posterior, very concave, cup shaped, bearing a small, exterior non-tubercular, inclined lip. Sucker eccentrically attached, in diameter equal to the transverse width of the urosome (not including branchiae). One pair of pigmented, irregular and asymmetrical eyes placed midlongitudinally on the anterior sucker. The strongly ventrally directed posterior sucker is centrally attached to the well-defined urosomal peduncle. Sucker is circular, very concave, with a contracted rim in some specimens, in others it is discoidal. On ventral surface are about 400 cupules. Diameter of sucker equal to transverse width of urosome + lateral branchiae. Beginning with XIIIa3 and ending with

[2] Drawn by J. H. Emerton.

XXIV, each ring bears, on its anterior margin, a pair of lateral, broad, foliaceous branchiae; a total of thirty-one pairs. Eleven pairs of diaphanous respiratory vesicles arranged segmentally, beginning with XIIIa2 and continuing posteriorly. Distinct prepuce is present. Five pairs of testes and a seminal vesicle. Stomach possesses paired lateral expansions between adjacent testes. Male gonopore on XIa2, female on XIIa2, separated by two annuli. Copulatory area, cocoon glands and ducts conspicuous. Intestine begins a short distance posterior to the last pair of testes and is characterized by four paired lateral, posteriorly directed expansions. Rectum large. Intestinal cecum coheres at five places. Anus opens posterior to XXVII.

Branchellion ravenelii varies in size[3] within fairly wide limits. The average length is about 15.0 mm (suckers included), while the average breadth is about 5.0 mm (branchiae included) or 2.5 mm (not including branchiae) at the widest place on the body. These measurements are not standard, however, since larger and smaller sexually mature specimens were observed.

EXTERNAL MORPHOLOGY

The description which follows is based on the dorsal aspect; body elongated, depressed, slightly convex dorsally, entirely flat ventrally. This cross-section picture is, however, more characteristic of the urosome than the trachelosome, which is more elliptical or cylindrical. The body is sharply divided into two distinct regions (Fig. 3); the first comprises about $\frac{1}{4}$ of the total length and is composed of twelve somites (twenty-rings), and the second comprises twenty-two somites (thirty-nine rings), representing the remaining $\frac{3}{4}$ of the total length. Of the twelve somites contained in the trachelosome, I–IV are absorbed by the anterior sucker, and from XXVIII–XXXIV of the urosome are absorbed by the posterior sucker, leaving twenty-three somites free in the body. The dorsal surface is void of neural annulus, metameric sensillae such as are found in *Branchellion torpedinis*.

Regarding the color of *B. ravenelii*, I can do nothing more than describe the condition of specimens preserved in alcohol. The ground color varies from whitish to gray, with gray perhaps predominating. If more color and/or color markings were present in life they have been removed by the preservative.

Trachelosomal Region. The trachelosomal region (Fig. 2) may be conveniently divided into the anterior sucker, pre-clitellum, and the clitellum. The sucker plus one small somite represents somites I–IV. The oral sucker is small as compared with the posterior, very concave, cup

[3] Fundamentally, size is of little value taxonomically. But it cannot be denied that the relationship of body width to length, the body proper length (exclusive of suckers), the features separating the trachelosome from the urosome, the over-all length (body proper + suckers), the separation and the absolute and relative development of the two suckers—all of these are deserving of consideration. In other words, these characteristics seem to be of greatest value when other related forms are being compared in detail and not indicated by linear dimensions only.

shaped, bearing a small, exterior non-tubercular, inclined lip. The sucker, which in diameter is equal to the transverse width of the urosome (exclusive of the branchiae), surrounds the mouth. A single pair of pigmented, irregular and asymmetrical eyes are placed mid-longitudinally on the sucker. Somite V is composed of three small annuli; somite VI, of three annuli slightly larger in both dimensions than those of V; somite VII, of a small anterior and a larger posterior annulus; somite VIII, of two large annuli, each being equal in length to the posterior annulus of VII; somite IX, of two incomplete annuli, the anterior being about twice the length of the posterior; somite X, of two complete annuli of which the posterior annulus is twice the length of the anterior and shows a faint constriction which probably represents the tendency to divide to form a primary somite of three annuli. The clitellar region is composed of somites XI and XII, each of which is composed of three approximately equal annuli. In somite XI, beginning with a1, a2, and a3 each shows a diminution in transverse width. In XII, annuli a1, a2, and a3 are approximately equal when measured cross-wise, but a1 is nearly equal to a2 + a3 when considered longitudinally. On a2 of XI the male gonopore opens, and on a2 of XII the female gonopore. Beginning with V and continuing through IX, the annuli, instead of being divided by straight transverse lines as is commonly the case, are separated by corrugated or serrated lines. The three annuli of XII and about half of XIa3 are covered by the pre-urosome.

As is evident from the above somite-annuli analysis, the somites do not show a definite graduated increase as to the number of annuli comprising the respective somites. They do, however, show a constant, uniform length increase, and beginning with somite VII each somite contains a neural ganglion which is located mid-longitudinally. Because the presence of a ganglion is the fundamental test of a segment, it seems that this is of greater significance and consequently should receive greater consideration than the mere somite-annuli arrangement. Although a constant somite-annuli increase does exist in some forms (i.e., GLOSSIPHONIIDAE), a non-uniform increase is not uncommon in certain PISCICOLIDAE; viz., *Pontobdella* spp., *Trachelobdella* spp., *Stibarobdella* sp. and others.

Urosomal Region. The posterior region which comprises about $\frac{3}{4}$ of the over-all length may be conveniently divided into the pre-urosome, urosome, and the posterior sucker. The sides of the body are parallel for their entire distance, with the exception that there is a slight narrowing anteriorly through the three anterior-most rings and posteriorly, beginning with XXIV, there is a more noticeable constriction, forming a peduncle to which the sucker is attached. There are twenty-two segments comprising the urosome which are divided into thirty-nine rings. Beginning

with XIIIa3 and ending with XXIV, each ring bears, on its anterior margin, a pair of lateral, broad, foliaceous branchiae which are almost half as long as the body is wide. Beginning with XIIIa2 and continuing posteriorly, eleven pairs of diaphanous respiratory vesicles are found. The first pair of vesicles occurs one annulus anterior to the first pair of branchiae, and all vesicles are located on the neural annulus, with the exception of the eleventh or last pair of vesicles. The second pair is in juxtaposition with the third pair of branchiae, the third with the sixth, and thereafter the vesicles occur in conjunction with every third pair of successive branchiae. The anus opens dorsally porterior to XXVII. The somites are of the primary or tri-annulate type, with the exception of XIII and those in the posterior region where somite-annulus diminution makes its appearance. Somite XIII is composed of two approximately equal annuli and two anterior, smaller equal rings the former two constitute a portion of the urosome, while the latter two form the pre-urosome or prepuce. The interpretation that the preputial fold is composed of two rings agrees with Apathy's interpretation (1888: 170) of the preputial fold in *Branchellion torpedinis*. However, he regarded the region in question as constituting a complete somite within itself. If one analyzes the neural annulus (the one which carries the respiratory vesicles) as the first annulus of the somite (current then) instead of the center of the tri-annulate somite (current now), *B. ravenelii* agrees exactly with *B. torpedinis* or vice versa.

Since the two rings composing the pre-urosome do not constitute a true part of a neuromere, gonomere and/or gastromere, it appears logical to consider them analogous to the first annulus of a typical tri-annulate somite, though somewhat atypical. If one considers this somite (XIII) analogous to the typical tri-annulate somite, there are ten such tri-annulate or primary somites which are followed by five comprising a lesser number of annuli. Somite XXIII is considered to be formed of two equal annuli; XXIV, XXV, XXVI, and XXVII are composed of a single annulus. The posterior sucker + one ring, which contains the posterior ganglionic mass, is assumed to represent seven somites. Therefore, the body is composed of thirty-four somites, the number composing a leech as determined by Moore and by Castle, whose conclusions were followed by subsequent workers.

The strongly developed posterior sucker, which is directed strongly ventrally, is centrally attached to the well-defined urosomal peduncle. This sucker is circular, much larger than the oral, very concave and exactly terminal, with a contracted rim in some specimens, while in others it is discoidal. On the ventral surface there exists about 400 cupules or secondary suckers.

DIGESTIVE SYSTEM

The rather small mouth opening is situated at the center of the anterior cupuliform sucker. The proboscis ends in VIII, where it receives the numerous ducts of the conspicuous esophageal glands, which are situated some distance anterior to the point of their emptying level. The proboscis sheath consists of a tube with thick and muscular walls and covers the distance from the mouth to the level where the esophageal ducts empty. It is partly surrounded by the large anterior ganglionic mass, and on each side the esophageal glands are linearly arranged. The proboscis sheath and proboscis is an adaptation of the pharynx, which has become highly muscular, the proboscis being free, protractile and thus capable of being thrust through the small oral opening in the leech's anterior sucker into the tissues of the host.

A narrow, thin-walled tube which is called the esophagus passes posteriorly from the proboscis to somite XIV, where it expands into the stomach. The stomach possesses four pairs of single digitate expansions known as the stomach diverticula, which exist between the five pairs of testes. Between each pair of testes, the stomach is constricted so as to fall within the space between the testes comprising the pair. The intestinal cecum, which continues posteriorly to XXIII/XXIV and is placed ventrally to the intestine, has no lateral diverticula. Apparently this intestinal cecum represents two fused ceca which cohere at five places and corresponds to the fourth stage in the development of cohesion from the primitive pair of ceca, as figured by Johansson (1896, Pl. VIII, Fig. 82) for *Piscicola geometra*. The first lacuna of the intestinal cecum is contained in XVIIIa2–a3, and the three following ones are located in successive somites in the same annular position. The fifth lacuna, however, is longer and consequently is contained in the first ring of XXIII, and in a2 and a3 of XXII.

The intestine is a moderately wide, medianly situated, longitudinal portion of the alimentary canal, beginning in somite XVIIIa1 and continuing through XXIIa2. Posteriorly the canal shows a wide expansion to form the rectum, which begins at XXIIa3. Its terminal part bends sharply upwards and discharges its contents through a dorsal and median anus, situated posterior to XXVII. The intestine is characterized by four pairs of lateral posteriorly directed, recurved expansions, which are articulated into a complicated series of folds. The lateral diverticula originate anteriorly at the mid-level of each of the first four intestinal cecal lacunae—or approximately at the level of the third annulus of the respective somites.

Thus the digestive system is composed of a number of parts which, for the sake of clarity, may be designated mouth, proboscis and proboscis sheath region (pharynx), esophagus, esophageal glands, stomach with

its diverticula, intestinal cecum, intestine with its diverticula, rectum, and anus.

REPRODUCTIVE SYSTEM

As is true of other leeches, *Branchellion ravenelii* is monoecious and the male and female gonads are paired. The reproductive system is of great systematic value because of the great variety exhibited in the details of the various parts.

If one examines one of these animals on the ventral surface, one sees very clearly a large orifice; it is the male gonopore and is situated at XIa 2. The female gonopore, a great deal smaller, cannot be clearly seen exteriorly because it is hidden by the integumentary fold which surrounds the genital region (clitellum) in the fashion of a prepuce, and is known as the pre-urosome.

Male Genitalia. There are five pairs of testes disposed between XIIIa3 and XVIIIa1, lateral to the stomach and between its lateral diverticula; i.e., intersegmentally, since the diverticula occur intrasegmentally. From each testis proceeds latero-anteriorly a small ciliated duct, the vas efferens, there being as many vasa efferentia as there are testes. Laterally from the posterior testis and proceeding anteriorly on each side, is the ciliated vas deferens, which in its course receives the remaining vasa efferentia. The vasa deferentia are in the region of the testes very fine tubes little larger than the vasa efferentia, but having a larger lumen.

Anterior to the first pair of testes, in XIIIa2, each vas deferens curves slightly inward, forms a short straight loop, then continues anteriorly through the clitellar region. At the level of the male gonopore (XIa2) the vasa deferentia curve abruptly outward and continue anteriorly before expanding into the paired seminal vesicles. It is in the seminal vesicles that the spermatozoa become cemented together in compact bundles, known as spermatophores, and are stored for future use. From each seminal vesicle an ejaculatory canal continues anterio-latero-ventrally, becomes thinner, and takes on several sinuosities before opening to the inside and anteriorly into the well developed, spacious, glandular terminal portion, or atrium. It is muscular and evidently protrusible.

Female Genitalia. The female reproductive organs consist of a pair of ovaries, swollen at their posterior part, and extending posteriorly to the posterior border of XIVa1. The oviducts are slender and unite in front of XIIa3 to form a common muscular oviductal gonopore opening in XIIa2.

A copulatory area does occur in *Branchellion ravenelii* very similar to that shown by Brumpt (1901:73) for *B. torpedinis*. In frontal and transverse sections this area is observed to begin posterior to the female gonopore and extend into the pre-urosome.

Cocoon Glands. These glands—which, however, are present in almost all HIRUDINEA,—show their greatest development in the PISCICOLIDAE. The prominent ducts of these glands (clitellar glands of Bourne) always open on the clitellum and are very conspicuous. The glands extend from the posterior limit of the clitellum to the region near the anus, forming a continuous layer beneath the longitudinal muscle layers and are imbedded in the dense body parenchyma. The glands are formed of spherical cells somewhat elongated in the direction of the body length.

NERVOUS SYSTEM

Inasmuch as the presence of a ganglion is the fundamental test of a segment, there are thirty-four ganglia in the central nervous system. Thus, the circumpharyngeal ganglionic mass contains six, and the posterior ganglionic mass contains seven fused ganglia. Twenty-one free single ganglia lie, metamerically disposed, within the limits of the somite in the ventral chain, between the anterior and posterior masses.

The ventral, median gangliated nerve-chain with its connecting commissures runs from the base of the urosomal peduncle to V and VI where the large cephalic ganglionic mass encircles the pharynx. The anterior ganglionic mass represents six closely joined neuromeres and the posterior ganglionic mass seven.

Throughout the middle portion of the body the nerve-chain ganglia are about equal distances apart, but toward the ends they are crowded closer together.

SUMMARY

Material collected from the coast of Florida has rèndered possible a detailed morphological study of *Branchellion ravenelii* (Girard, 1850), including for the first time the internal morphology of this species. Verrill's leech collection, in which was one specimen labeled *Phyllobranchus ravenelii* = *Branchellion ravenelii,* has been available for comparison.

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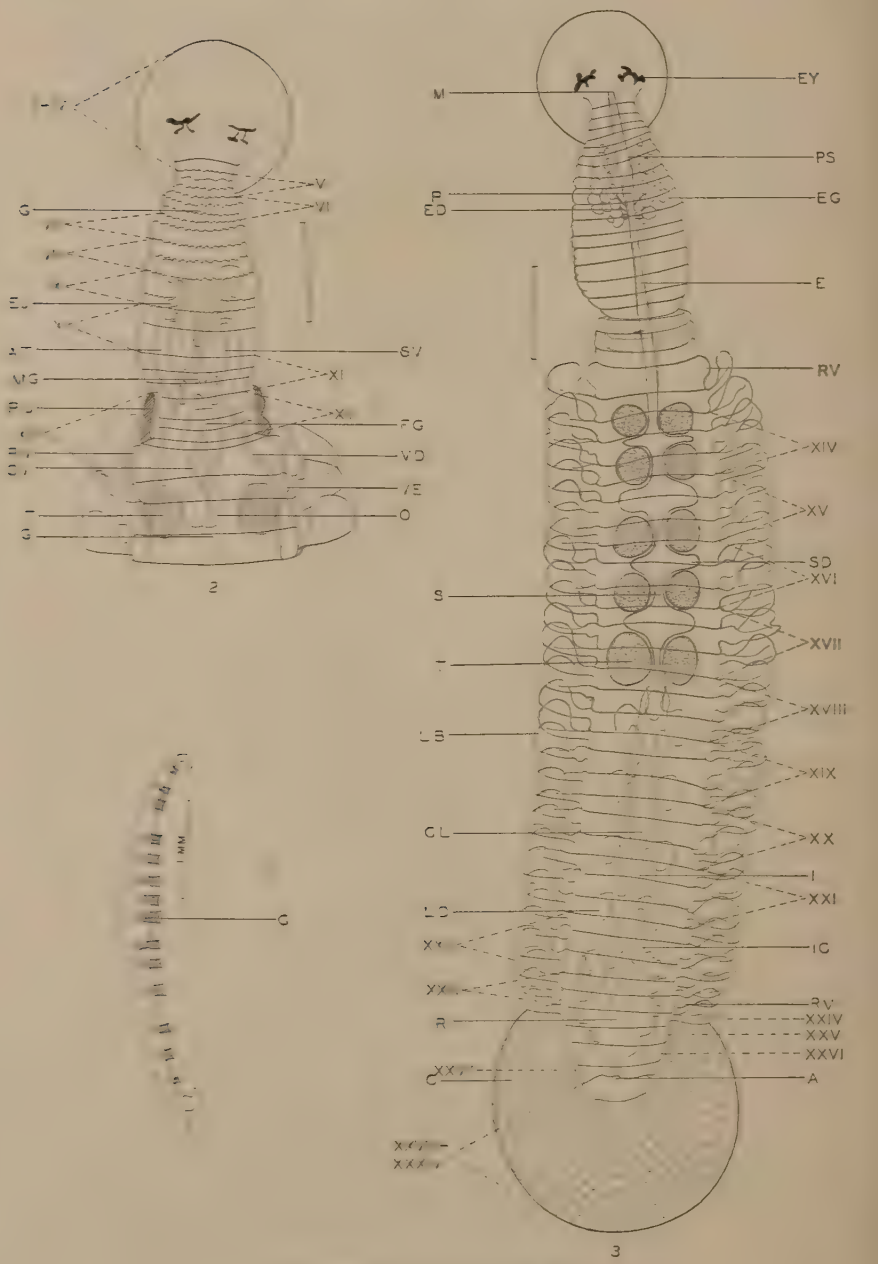
EXPLANATION OF PLATE

A—anus	G—ganglion	PS—proboscis sheath
AT—atrium	I—intestine	PU—preurosome
C—cupule	IC—intestinal cecum	R—rectum
CL—cecal lacuna	LB—lateral branchus	RV—respiratory vesicle
E—esophagus	LD—lateral diverticula	S—stomach
ED—esophageal gland duct	M—mouth opening	SD—stomach diverticula
EG—esophageal gland	MG—male gonopore	SV—seminal vesicle
EJ—ejaculatory duct	O—ovary	T—testis
EY—eye	OV—oviduct	VD—vas deferens
FG—female gonopore	P—proboscis	VE—vas efferens

Each scale line represents 1 mm.

FIG. 1. Transverse section through the posterior sucker, showing cupules.
FIG. 2. Anterior region, showing in detail the reproductive systems and annuli-somite arrangement of the trachelosome (Dorsal view).
FIG. 3. Dorsal drawing of entire animal, showing in detail the digestive system.



Branchellion ravenelii (Girard, 1850)

STUDIES ON THE LIFE HISTORY OF THE ANOPLOCEPHALINE CESTODES OF HARES AND RABBITS*

HORACE W. STUNKARD
New York University

The anoplocephaline cestodes of rabbits and hares are distributed among the genera *Andrya, Cittotaenia, Paranoplocephala* and *Schizotaenia*. Reviews of the described species were published by Baer (1927) and Arnold (1938). The latter author showed that the cestodes from leporine hosts in North America are distinct from those of Europe. His findings are not surprising, since the hares of the two continents belong to different species and the rabbits to different genera. Two species of *Andrya* have been described from European hares and rabbits and although the genus is represented in North America, no species have been reported from leporine hosts in the Western Hemisphere. Similarly *Paranoplocephala wimerosa*, described from hares and rabbits in Europe, is the only species recorded from these hosts, although additional species of the genus are known to infect other animals in different parts of the world. The genus *Schizotaenia* appears to be limited to the Western Hemisphere. Although *S. americana* occurs in hares in North America, none of the other species in either North or South America has been reported from leporine hosts. On the other hand, the genus *Cittotaenia* has representatives in both European and North American rabbits and hares: *C. ctenoides, C. denticulata* and *C. pectinata* in Europe and *C. variabilis, C. perplexa* and *C. pectinata americana* in North America. Incidence of infection with European species of *Cittotaenia* was reported by Riehm (1881), John (1926), Rees (1933a), Stunkard (1934) and Evans (1940). John gave a detailed description of *C. denticulata* and Rees of *C. pectinata;* Evans reported the incidence of the two species for each month of the year. It is interesting to note that these authors found *C. pectinata* and *C. denticulata* very common in wild rabbits of Great Britain, but did not report *C. ctenoides*. In Germany, Riehm found *C. ctenoides* the most common species in rabbits, while *C. pectinata* was rare in rabbits and common in hares. My records agree with those of Riehm.

The life history of the anoplocephaline cestodes, which for more than

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a half century has been one of the most enigmatic problems in parasitology, is now at least partially disclosed. Previous studies on the life cycle of these tapeworms were reviewed by Stunkard (1934). That paper recorded experiments conducted during the academic year 1931–1932 at the Institut für Schiffs- und Tropenkrankheiten in an attempt to discover the life cycle of the cestodes of rabbits. These animals appeared to be ideally suited for such investigation. Rabbits are small, can be maintained in close confinement, and their food can be examined more easily for infective agents than that of larger herbivores. The wild rabbits of Northern Germany are heavily infected with tapeworms and since the domestic variety was derived from the wild European rabbit and is susceptible to infection with certain of the cestodes of wild rabbits, the selected location and species afforded an opportunity to pursue the study under favorable and well controlled conditions. Results of the experiments demonstrated that the onchospheres of *Cittotaenia* complete their development in the egg and that they do not become infective for the final host. Since direct infection in these species does not take place, an intermediate host must be necessary for the completion of the cycle. From bionomic data, the characteristic features of the intermediate host were clearly delineated.

Utilizing the information acquired from the Hamburg experience, study of the life history of anoplocephaline cestodes was continued in New York and the life cycle of *Moniezia expansa* was worked out and reported (Stunkard, 1937a, 1937b, 1937c). Galumnid mites were found to serve as intermediate hosts and the results have been confirmed by Stoll (1938), Krull (1939a) and Shorb (1939). More complete accounts were presented by Stunkard (1939a, 1939b). The life cycle of a second anoplocephaline species, *Bertiella studeri* a parasite of monkeys and man, was reported by Stunkard (1939c, 1940a, 1940b). The life history of a third species, *Cittotaenia ctenoides* from European rabbits, was reported by Stunkard (1939d). The present paper presents a more complete account of the development of *C. ctenoides,* and observations on the life cycle of two other species, *C. denticulata* and *C. pectinata.* So far as known, all of these anoplocephaline cestodes employ oribatid mites as intermediate hosts; consequently it becomes increasingly probable that other members of the family have a similar life cycle.

EXPERIMENTAL METHODS AND RESULTS

It was a great satisfaction to return to Hamburg in the summer of 1938 to continue the studies begun there seven years before. The discovery of the life cycle of *Moniezia expansa,* which indicated that oribatid mites may serve as intermediate hosts of the rabbit cestodes, and the experience of the previous year in the "Tropeninstitut" were of value in

outlining the present investigation. Wild rabbits are not readily obtained in Hamburg during the summer and early autumn. Accordingly, it seemed wise to devote this period to the collection and dissection of mites, in an effort to discover cysticercoids of natural infection. Such cysticercoids could be fed to young, laboratory-raised domestic rabbits and if the larvae developed to sexual maturity it would be possible to identify both the cestodes and their field hosts. Information concerning the natural intermediate hosts would be very helpful in planning experimental infections later in the season when wild rabbits are abundant in the markets and eggs from identified cestodes can be obtained in large numbers.

Representatives of the mites common in the Hamburg area were identified by Dr. Max Sellnick of Königsberg. Grateful acknowledgment is made for his kindness in determining the material. Although identified specimens were used for comparison, it is possible that some of the mites examined for natural infections and others used later in feeding experiments were not correctly identified, because of the rapidity with which mites were dissected and the inherent difficulty of specific determination by one who is not a specialist in the group.

Work on the life cycle of the rabbit cestodes was impeded during the fall by experiments conducted simultaneously to determine the life history of *Bertiella studeri*. In the latter studies (Stunkard, 1940a), eggs of the parasite were fed to many species of mites, collected in areas where they would not ordinarily be exposed to eggs of anoplocephaline tapeworms. The discovery that the onchospheres of *Bertiella* would emerge in the intestine of various mites and begin development in the body cavity, suggested that more than one species might be able to serve as intermediate hosts of this and other members of the family ANOPLOCEPHALIDAE.

Experimental Infection of Rabbits

Mites were collected in the regions of Wohldorf and Duvestedt and dissected in the attempt to obtain natural infections with the cysticercoids of rabbit cestodes. Incidence of infection in mites would depend naturally on the number of rabbits in the district and on the incidence and intensity of infection among them. Consequently, mites were collected in areas where rabbit feces were abundant and yielded large numbers of cestode eggs. The collections were made after light rains or in the early morning or late evening. Larger numbers of mites could be collected on dark, cool, humid days. A moist area was selected where the grass was at least 10 cm high. With a sharp garden tool, the roots of the grass were cut 2 to 3 cm below the surface of the ground and pieces of sod 10 to 20 cm in diameter were taken. These samples of earth, grass roots and grass were placed in closed containers and transported to the labora-

tory. The material was then screened through a fine sieve on a large piece of white paper which was brightly lighted. The light stimulated the mites to move and they were picked up with a moist, camel's-hair brush. Krull (1939b) described the method employed by him for the collection of oribatid mites. Grass was cut in the field and transferred to the laboratory where the mites were washed from it by warm or cold water. Krull stated, "It appears that the actual number of mites on grass varies considerably, and that there are times, even in spring, when no mites can be recovered." He noted also, "They appear to leave the grass when there is a high wind." I have observed that mites often drop from the grass when it is disturbed and the method of Krull would probably recover only a fraction of the specimens present in an area. In the same paper Krull reported that mites can not ingest *Moniezia* eggs, but that the mite makes a hole in the shell and ingests the contents. Eggs to be accessible must be well anchored; otherwise they were merely pushed around. This observation by Krull is supported by my finding (January 14, 1939) of six pyriform apparatuses with larvae inside them but without egg shells, in the intestine of a specimen of *Liacarus coracinus* which had been fed *Cittotaenia denticulata* eggs the previous day.

Over six thousand mites, belonging to thirty-eight different species, were dissected between July 27th and November 1st. On September 8th, in a specimen of *Scutovertex minutus,* I found a cysticercoid (Fig. 25) which closely resembled that of *Moniezia expansa*. Examined in a drop of water without a coverglass, it measured 0.216 mm in width and 0.18 mm in length. The apical end was slightly flattened and there was a small fibrous mass, the remains of the cercomere, attached at the base of the cyst. The cyst wall was firm and fibrous, and calcareous bodies were loose in the cavity between it and the scolex. The scolex was 0.13 and the suckers 0.06–0.07 mm in diameter. Movement of the larva and suckers was noted. A small hole was made with a needle in a grain of wheat, the larva was placed in it and then fed to a half-grown rabbit, No. 1. The feces of the rabbit were examined twice each week. They were negative until November 24th, when seven cestode eggs were recovered in the examination of forty fresh pellets. A few eggs were found in feces collected on November 25th, and they were abundant in feces collected November 26th. The rabbit was killed on this date and a single, fully developed specimen of *C. ctenoides* was found in the intestine. It was very active, and after removal of the sixteen terminal proglottids, the worm was extended and fixed. Eleven weeks had elapsed from the time the larva was ingested until eggs were found in the feces. A brief preliminary note, (Stunkard, 1939d) announced the life cycle of *C. ctenoides*.

On September 19th, dissection of a specimen of *Scheloribates laeviga-*

tus yielded a cysticercoid. It was almost spherical, 0.188 mm in diameter, was slightly flattened at the apical end and bore a very short, stalk-like cercomere. The cyst wall appeared to be a little thicker than the one found on September 8th, and there were more concretionary bodies between the larval scolex and the cyst wall. The larva was active within the cyst. The cysticercoid was placed in a grain of wheat and fed to a half-grown rabbit, No. 2. The feces of the animal were examined twice a week for four months and no cestode eggs were found. At that time it was killed and no tapeworm was present.

On September 19th, two cysticercoids were removed from a specimen of *Scutovertex minutus*. They were similar to the one found on September 8th, but one was slightly smaller. The cyst wall of the larger one was injured during the dissection; both were fed to the same young rabbit, No. 3. Biweekly examination of the feces of this rabbit for a four month period did not yield any cestode eggs and it was negative when dissected at that time.

Other mites from infected areas were dissected during the winter and large numbers were examined in the spring of 1939. On May 8th, a cysticercoid which measured 0.216 by 0.2 mm was found in *Pelops tardus*. It contained an active larva and was fed to a young rabbit, No. 8. The rabbit was negative when dissected on May 30th. Since the three larvae found on September 19th failed to develop to sexual maturity and the one taken on May 8th was not recovered, their specific identity remains in question. It is probable that they were larvae of *Cittotaenia,* although deer range over the area and eggs of anoplocephaline tapeworms, probably *Moniezia,* have been recovered from the feces of deer.

Experimental Infection of Mites

The second phase of the investigation was concerned with attempts to infect mites by feeding them eggs of rabbit cestodes. In the feeding experiments, only eggs from identified cestodes were used. To secure eggs for this purpose, 20 hares and 93 rabbits were dissected during the autumn and winter. None of the hares was infected and no specimens of *C. pectinata* were found. This result was unexpected since 10 of 56 hares examined during the winter of 1931–1932 harbored *C. pectinata* and the worms were often present in large numbers, (Stunkard, 1934). Of the 93 rabbits examined, 63 yielded specimens of *C. ctenoides,* 8 contained specimens of *C. denticulata,* and 3 were infected with *Andrya cuniculi.* No mature specimens of *A. cuniculi* were found and since no eggs of *C. pectinata* or *A. cuniculi* were available, the experiments were limited to *C. ctenoides* and *C. denticulata.* Usually one or two, but never more than two mature specimens were found in a rabbit; often several immature specimens were present together with one or two ma-

ture ones. The dissection of hares and rabbits during the two seasons, 1931–1932 and 1938–1939, provided many small, immature specimens of all three species of *Cittotaenia* and made it possible to trace the successive stages of their development in the final host.

For experimental infection of intermediate hosts, mites were collected from areas to which rabbits would not have access. Representatives of all the common species were used in the experiments. The mites were maintained in culture dishes. Usually 200 to 300 mites of various species were placed in each culture. The dishes were kept at room temperature, away from direct light and provided with abundant food and moisture. When mold growth became excessive, the mites were transferred to clean dishes. Eggs of *C. ctenoides* or *C. denticulata* were added to culture dishes as recorded below. When the mites were transferred to clean dishes, which was necessary every few days at least and often daily, most of the cestode eggs were left behind. Since the cestode eggs were added to the mite cultures and allowed to remain there, it is impossible to tell when they were eaten and exact dates of infection are not available. Presumably the eggs were ingested at different times and since the larvae remain viable in the eggs for weeks in moist chambers, repeated infection probably occurred. The number of available eggs was rapidly diminished, however, by the successive transfers of the mites to clean nests. Some eggs were attached to the bodies of the mites and others were dried on pieces of filter paper and transferred with them, but most of the infections were probably acquired during the first few days. Since the object of the experiment was to complete the cycle in the intermediate host, only those mites which died in the cultures were dissected. Often when dead mites were removed and examined, they were so dried internally that larvae, if present, would have disintegrated. The larvae are exceedingly delicate and changes in osmotic relations cause them to die and decompose. Many of the larvae were photographed alive, but they were so fragile that they often disintegrated before the photograph could be made. The figures on plates I and II record successive stages in development, but blistering and other evidences of disorganization appear in the figures. The dates of exposure and dissection, names of hosts, with number and size of the larvae recovered, are presented in the following tables. Only those dissections which yielded larvae are recorded. The mites were dissected in a drop of water or Ringer's solution and sometimes two or more mites of a single species and from the same culture were dissected in a single drop. In such instances, it was not possible to determine with certainty the number of larvae which came from each mite. In the first series (Cultures I to III), begun on October 12th, the eggs came from proglottids which had been cut from a worm and the dissection of 50 mites during the first two weeks yielded

only two infections. It was feared that many of the larvae were not infective and so other eggs, from detached proglottids, were added on October 26th. With the procedure followed, it was impossible to determine precisely when the eggs were eaten and allowance for this fact must be made in the interpretation of the results.

Cultures I–III, exposed to eggs of *C. ctenoides*, October 12 and 26, 1938
Dates of dissections and results

October 17. Two *Scutovertex minutus:* recovered 2 living onchospheres; 0.025 × 0.022, and 0.022 × 0.02 mm.

October 22. Two nymphs, *Galumna* sp.: recovered 6 larvae; largest 0.04 mm in diameter, one 0.03 mm, the others smaller.

November 22. Three *Scheloribates laevigatus:* recovered 5 larvae; 0.065, 0.05, 0.042, 0.038. and 0.032 mm in diameter. The 3 largest ones had small cavities.

November 24. One *Notaspis coleoptratus:* recovered 5 larvae; 0.06, 0.048, 0.041, 0.036, and 0.032 mm in diameter.

November 26. One *Scutovertex minutus:* recovered 5 larvae; the largest (Fig. 2) was 0.095 mm in diameter, the smallest (Fig. 1) was merely an onchosphere, 0.028 × 0.025 mm.

Two *Scheloribates laevigatus:* recovered 3 larvae; 0.036 mm in diameter, 0.037 × 0.034, 0.022 × 0.02 mm.

One *Trichoribates incisellus:* recovered 3 larvae; 0.07 × 0.07, 0.038 × 0.034, and 0.024 × 0.022 mm.

November 28. One *Scheloribates laevigatus:* recovered 3 larvae; 0.075 × 0.075, 0.032 × 0.028, and 0.022 × 0.02 mm.

December 5. One *Galumna obvious:* recovered 3 larvae; largest 0.032 × 0.029 mm. One *Galumna nervosus:* recovered 2 larvae, largest 0.037 × 0.035 mm.

December 10. One *Scheloribates laevigatus:* recovered 4 larvae; 0.15 × 0.13, 0.136 × 0.1, 0.1 × 0.09, and 0.028 × 0.026 mm.

December 14. One *Scheloribates laevigatus:* recovered 4 larvae; largest 0.035 × 0.034, one 0.03 × 0.027 mm, others smaller, size not recorded.

One *Pelops acromius:* recovered 1 larva; a young cysticercoid, cyst 0.2 × 0.18 mm, cercomere 0.25 mm long and 0.08 mm wide at the base, with hooks at the distal end of the cercomere. On application of a coverglass the cyst opened and contents were everted (Fig. 9); the sucker pads were 0.055 × 0.048 mm.

December 17. One *Galumna obvious:* recovered 14 larvae; 2 young cysticercoids, in one the cyst was 0.13 mm in diameter, the cercomere 0.32 mm long and 0.07 mm wide at the base, with hooks at the tip. The other was about the same size. One pyriform larva measured 0.33 × 0.18 mm, others 0.23 × 0.16 mm, 0.21 × 0.15 mm, 0.14 × 0.13, 0.1 × 0.1; the smallest larva measured 0.065 mm in diameter. The young cysticercoids everted in Ringer's solution before a coverglass was added and all the larvae disintegrated within 30 minutes.

December 19. One *Galumna obvious:* recovered 10 larvae; 2 young cysticercoids with cercomeres about 0.3 mm long (Figs. 12, 13, 14); the cyst of one (Fig. 14) 0.2 × 0.19 mm, the other (Fig. 13) 0.17 × 0.17 mm. One larva was pyriform with cercomere (Fig. 8), the body 0.31 mm long with cercomere of almost equal length, the sucker discs near the anterior end and hooks in tip of cercomere were clearly visible. Two were vermiform, one (Figs. 6, 12) 0.413 mm long and 0.18 mm in greatest width, the other 0.4 × 0.19 mm. The other larvae were smaller, the smallest 0.06 mm in diameter.

December 20. One *Galumna obvious:* recovered 10 larvae; the largest was almost identical in size and appearance with the one shown in Fig. 8, one was pyriform with the beginning of cercomere constriction, the body 0.3 mm long (Fig. 7). Two were oval, 0.24 × 0.18 mm, and the other (Fig. 4) 0.2 × 0.16 mm. The other larvae were smaller. One *Galumna obvious:* recovered 1 cysticercoid with small atrophied cercomere. It measured 0.18 × 0.17 and was fed to a young domestic rabbit, No. 4.

December 21. One *Galumna obvious:* recovered 12 larvae; 6 cysticercoids 0.17–0.2 mm in diameter, all with cercomeres, some slightly longer and others shorter than the diameter of the cysts. All were fed to rabbit No. 4. There were 3 vermiform larvae, 0.28 to 0.36 mm in length, similar in appearance to Fig. 6. Three larvae were oval, 0.2 to 0.36 mm in length and corresponded in development to stages shown in Figs. 4 and 5.

December 30. One *Galumna obvious:* recovered 3 larvae; the largest was pyriform and without coverglass measured 0.137 × 0.12 mm, the smallest was spherical and about one-half as large.

December 31. One *Liacarus coracinus:* recovered 3 larvae; the largest had pyriform body 0.15 mm long and 0.12 mm wide, the sucker pads were visible, the cercomere was 0.24 mm long and 0.07 mm wide at the base. The other larvae were oval but blistered early and no measurements were recorded.

January 3. One *Scutovertex minutus:* recovered 3 cysticercoids, 0.21 × 0.18 mm with cercomeres. They were dried and somewhat flattened, fed to rabbit No. 4.

January 5. One *Liacarus coracinus:* recovered 4 larvae; the largest (Fig. 5) was 0.4 × 0.266 mm but shortened before the photograph was taken; note the germinal cells in the specimen. The other larvae were oval, like Fig. 4.

January 6. One *Liacarus coracinus:* recovered 5 larvae; 3 young cysticercoids with cercomeres about as long as the diameter of the cysts, fed to rabbit No. 4. One larva was pyriform with beginnings of cercomere constriction, one was vermiform.

January 6. One *Galumna obvious:* recovered 8 larvae; 1 cysticercoid was fed to rabbit No. 4, 3 pyriform larvae with cercomeres like Figs. 7 and 8, 4 spherical to oval larvae without cercomeres.

Culture IV, exposed to eggs of *C. ctenoides,* November 21, 1938

November 29. *Scheloribates laevigatus:* 1 larva; 0.031 × 0.027 mm.

December 14. *Trichoribates incisellus:* 6 larvae; largest 0.136 × 0.12 mm, another about the same size, 1 measured 0.11 × 0.1 mm (Fig. 3, note the blister and body cavity), the smallest was 0.026 × 0.023 mm.

December 27. *Cepheus cepheiformis:* 7 larvae; largest 0.11 mm in diameter, smallest 0.07 mm in diameter, all were spherical with cavities.

Notaspis coleoptratus: 12 larvae; largest spherical 0.087 mm, smallest 0.034 × 0.03 mm.

Notaspis coleoptratus: 6 larvae; largest spherical 0.08 mm, smallest 0.032 × 0.03 mm.

December 28. *Notaspis coleoptratus:* 3 larvae; the largest pyriform changed to oval 0.13 × 0.094 mm, second spherical 0.087 mm, smallest 0.05 mm in diameter.

January 2. *Scheloribates laevigatus:* 3 larvae; spherical 0.072, 0.052, and 0.042 mm.

January 23. *Notaspis coleoptratus:* 4 larvae; 3 cysticercoids, cysts 0.14, 0.13, and 0.12 mm, with cercomeres about as long as the diameter of the cysts, 1 oval larva 0.146 × 0.128 mm.

Scheloribates laevigatus (laboratory raised specimen): 6 larvae; the largest vermiform 0.288 mm long × 0.1 mm wide, the second measured 0.2 × 0.13 mm, the smallest was spherical 0.09 mm in diameter.

February 8. *Liebstadia similis:* 2 cysticercoids, cysts 0.14 × 0.13 mm, with cercomeres 0.1 mm long and 0.015 mm wide at the base, fed to rabbit No. 5.

Culture V, laboratory raised *Scheloribates laevigatus* exposed to eggs of *C. ctenoides,* November 23, 1938

December 2. All mites dead, dissection of 2 yielded 8 larvae, all onchospheres.

Culture VI, exposed Nov. 30, 1938 to eggs of *C. ctenoides* taken from the worm raised in experimentally infected rabbit, No. 1

December 10. *Scutovertex minutus:* 2 larvae; onchospheres.

December 17. *Liacarus coracinus:* 3 larvae; largest spherical 0.06 mm, smallest 0.04 mm in diameter.

December 21. *Scheloribates laevigatus* (laboratory raised specimen): 3 larvae; largest 0.036 × 0.034 mm.
January 2. *Cepheus cepheiformis:* 1 larva; spherical 0.11 mm in diameter.
January 4. *Cepheus cepheiformis:* 6 larvae; largest spherical 0.095 mm with conspicuous cavity, smallest 0.04 mm in diameter.
January 9. *Notaspis coleoptratus:* 4 larvae; largest pyriform 0.237 × 0.1 mm, smallest 0.06 mm in diameter.
January 10. *Cepheus cepheiformis:* 2 larvae; oval, same size, 0.18 × 0.12 mm.
February 8. *Liacarus coracinus:* 1 cysticercoid 0.155 × 0.14 mm, with cercomere about as long as the diameter of the cyst, fed to rabbit No. 6.
February 10. *Xenillus tegeocranus:* 11 larvae; cysticercoids (Fig. 11) largest 0.18 × 0.15, smallest 0.1 × 0.1 mm, all fed to rabbit No. 6.
February 13. *Xenillus tegeocranus:* 9 cysticercoids, largest 0.18 mm, smallest 0.13 × 0.12 mm, the specimens were dried and the cercomeres could not be measured. They were fed to rabbit No. 6.

Culture VII, exposed to eggs of *C. denticulata,* November 10, 1938

December 12. *Xenillus tegeocranus:* 1 larva; 0.034 × 0.03 mm.
December 14. *Scutovertex minutus:* 4 larvae; spherical to oval, largest 0.12 × 0.11 mm, smallest 0.06 mm in diameter.
December 15. *Cepheus cepheiformis:* 6 larvae; largest vermiform 0.275 × 0.12 mm, 1 spherical 0.135 mm with a large cavity, others smaller.
December 17. *Trichoribates incisellus:* 2 larvae; 0.1 × 0.085, and 0.1 × 0.1 mm.
December 19. *Scutovertex minutus:* 5 larvae; 2 were pyriform 0.18 × 0.082 with the beginning of cercomeres, 2 were oval 0.09 × 0.08 mm, the other was oval 0.122 × 0.094 mm.
December 28. *Scheloribates laevigatus:* 1 larva; spherical 0.1 mm, large cavity.
January 2. *Xenillus tegeocranus:* 1 larva; an onchosphere.
January 4. *Scutovertex minutus:* 1 larva; vermiform 0.2–0.275 × 0.1 mm.
January 13. *Scutovertex minutus:* 2 larvae; 0.11 × 0.095, and 0.09 × 0.09.
January 31. *Scutovertex minutus:* 2 larvae; a cysticercoid, cyst 0.19 × 0.18 mm, with cercomere 0.1 mm long, anterior end of larva everted in Ringer's solution before it could be photographed, the other larva was vermiform 0.38 mm. long and 0.1 mm in greatest width.
February 9. *Scutovertex minutus:* 2 cysticercoids; 0.15 × 0.13 mm. One is shown in Fig. 10. Both were fed to a young rabbit, No. 7.

Culture VIII, exposed to eggs of *C. denticulata,* January 13, 1939

January 14. *Liacarus coracinus:* 6 pyriform apparatuses were found in the intestine of the mite; they contained onchospheres but no shells were found.
February 8. *Liacarus coracinus:* 1 larva; 0.075 × 0.07 mm, cells of two sizes, 6 hooks present, larva disintegrated in a few minutes.
February 10. *Scutovertex minutus:* 8 larvae; spherical 0.05 to 0.1 mm in diameter.
February 17. *Scutovertex minutus:* 6 larvae; spherical 0.05 to 0.09 mm in diameter.

Culture IX, exposed to eggs of *C. denticulata,* February 4, 1939

May 12. *Scutovertex minutus:* 2 larvae; a cysticercoid 0.2 mm in diameter with a shriveled cercomere, fed to rabbit No. 9; also a pyriform larva, body 0.216 mm long and 0.086 mm wide with a cercomere 0.1 mm. long.

Development of *C. ctenoides* was observed in eleven species of mites and cysticercoids were recovered from seven different species. About ten weeks are required for the larvae to reach the cystic stage in a mite. Since development appeared to be progressing normally in other mites which became infected but died, it is possible that these larvae might have

completed their development if their hosts had lived long enough. Development of *C. denticulata* occurred in six of the eleven species, although only *Scutovertex minutus* lived long enough for cysticercoids to be produced. It is noteworthy that infections were obtained only in oribatid mites. Present results indicate that these mites are the natural hosts of the anoplocephaline tapeworms, and that the host specificity is not at all rigid.

None of the rabbits, fed cysticercoids from experimentally infected mites, passed cestode eggs in the feces and all were negative when sacrificed and examined later. It is probable that the larvae, when ingested, were not yet infective. A further possibility exists, viz., that the domestic rabbit is a less favorable host than the wild variety. This explanation is suggested by the fact that only one of five cysticercoids from naturally infected mites developed in domestic rabbits. The explanation is not supported, however, by certain other experiments. Krull (1939a) fed five cysticercoids of *Moniezia expansa* to a lamb which on post-mortem examination yielded only two worms. Shorb (1939) recovered only 52 worms from the feeding of 468 cysticercoids of *M. expansa*. Indeed, one of the lambs, which received 203 cysticercoids, apparently did not become infected. It is possible that, in the natural hosts, only a few of the ingested cysticercoids develop to sexual maturity. An observation of Krull (1939a) bears on the question of susceptibility of the domestic rabbit. He reported that three of five rabbits became infected with species of *Cittotaenia* as a result of being kept on a plot near the shore of a pond where the mite *Galumna emarginata* was present; each infected rabbit harbored two immature tapeworms. This report confirms that of Honess (1935) who infected the domestic rabbit by feeding grass from an area frequented by wild rabbits.

DESCRIPTION OF STAGES IN DEVELOPMENT

Specific descriptions of *C. ctenoides, C. denticulata* and *C. pectinata* have been presented by Arnold (1938) and previous authors, so further details concerning the sexually mature worms are not essential in the present paper.

The eggs of *C. pectinata* were described by Rees (1933a) and the eggs and onchospheres of other European species by Stunkard (1934). In that report Stunkard noted that the eggs of *C. pectinata, C. denticulata* and *C. ctenoides* vary in size and appearance, depending on their condition at the time of examination, and that it had been impossible to find differential features which could be used to identify eggs in the absence of records. In the present study, eggs of only *C. ctenoides* and *C. denticulata* have been available. Although their size ranges overlap, eggs of *C. denticulata* average somewhat smaller than those of *C. ctenoides;*

furthermore as a rule, in fresh eggs of *C. denticulata* the horns of the pyriform apparatus are shorter, they taper more rapidly, and more frequently the tips are crossed. In both species, the appearance of the eggs changes markedly as the shell is formed and consolidated. These features are represented in Figs. 15 and 16.

To secure cestode eggs for the experimental infection of mites, gravid proglottids were sometimes removed from entire worms, but often the terminal segments contained few or only immature eggs. Such eggs were usually irregular in shape and had either no shell or one that was soft and hyaline. Although they contained motile onchospheres, it is doubtful whether they were infective. To obtain more mature eggs, the intestines of rabbits were examined throughout their length and often single or groups of detached proglottids were taken from the cecum and large intestine. These proglottids were sometimes full of shelled eggs and occasionally the eggs had been shed from them. Since mature specimens of more than one species of *Cittotaenia* were never encountered in the same intestine, there was no possibility for mistaken identity of detached proglottids. When gravid segments were pressed, the eggs poured out on both sides. They were embedded in the uterine secretion which adhered in whitish strings, vermiform in appearance, and as much as 5 mm in length. No preformed uterine pores, as described by Baylis (1934) for certain anoplocephaline species, have been observed in *Cittotaenia;* on the contrary, the lateral walls of the proglottids disintegrate and rupture as described by Stunkard (1940a) in *Bertiella studeri.* In water or after alternate drying and moistening, the eggs slowly separated and the shells became more compact.

In their early stages, eggs of *C. ctenoides* have no shell; they are bounded by a membrane, are spherical to oval with flattened areas where they have been compressed, and measure from 0.05 to 0.09 mm in diameter. There is an eccentric vesicle, 0.025 to 0.035 mm in diameter, attached to the external membrane and suspended in a fluid matrix. This matrix contains small granules or globules which manifest Brownian movement. The vesicle contains an onchosphere, the hooks of which may move slowly. This vesicle transforms into the cornuate pyriform apparatus, and a shell is deposited later on the external membrane. The shell is formed from the uterine secretion and at one stage appears thin and granular. At other times it consists of a series of transparent concentric rings. Such an egg is shown in Fig. 15. Older eggs have firm, hard shells, as described by Stunkard (1934). Although there may be larger and smaller ones, most of the eggs fall in the size range 0.075 to 0.085 mm in diameter. The pyriform apparatus is almost as long as the diameter of the egg, 0.021–0.025 mm wide at the base, with uniformly tapering horns. The onchospheres measure 0.019–0.021 mm in diameter

and their hooks are 0.010–0.011 mm long. Like those of *C. ctenoides*, young eggs of *C. denticulata* have no shell; indeed, eggs removed from proglottids seldom have shells. The outer covering of the egg is membranous, and the eggs, enclosed in a jelly-like mass, are arranged in irregular rows. The jelly mass is extruded with the eggs and later splits between them, contributing to the formation of the shell. Each egg is situated in a polyhedral mass which later shrinks and tends to become spherical. Usually the proglottids are shed before the eggs are shelled, but they are probably infective at this time. On November 10, 1938, a group of three proglottids was removed from the terminal part of the large intestine of a rabbit. The eggs pressed from them had only membranous coverings and were embedded in the jelly-like substance. These eggs were fed to mites in the experiment recorded earlier (Culture VII) and produced infections, although it is possible that they aged somewhat in the moist chambers before they were eaten.

No constant differences were noted between the onchospheres of *C. ctenoides* and *C. denticulata*. The experiments demonstrate that the larvae of both species may begin development in any one of a number of different oribatid mites and it is probable that development may be completed in more than one species of intermediate host. Early developmental stages of the two species are indistinguishable, and so only one species is represented in the figures of Plate I. Development of *Cittotaenia* in the intermediate host is very similar to that of *Moniezia* as described by Stunkard (1939a).

The onchospheres are active in the body cavity of the mite for two or three weeks. At the time of infection each onchosphere contains ten to fourteen large, germinal cells. In the haemocoele of the mite, the germinal cells multiply and the larva increases in size. Meanwhile the organization of the onchosphere is lost, its muscles atrophy, the hooks lose their regular positions and become functionless, and most of the mesenchymal cells disintegrate. The larva loses its bilateral character and becomes spherical and immobile. It consists of a mass of cells with an irregularly-shaped cavity on the side which bears the hooks. Such spherical larvae (Figs. 2, 3, 18, 19) measure from 0.04 to 0.11 mm in diameter and about four weeks are required to reach the larger size. The spherical stage is followed by one in which the larva becomes elongate and oval or pyriform (Figs. 4, 5, 20). As development proceeds the larvae become more elongate and vermiform, calcareous bodies appear in the parenchyma, muscles differentiate in the body wall and faint uncoordinated movements are discernible (Figs. 6, 21). The end opposite the hooks is much wider than the other and, with further development of the muscles, movements become more regular and contraction of the circular fibers may produce a segmented appearance. Such larvae may

extend to a length of 0.4 mm. Later a definite zone of contraction cuts off a cercomere portion from the body proper (Fig. 7). This process continues until a condition represented by Fig. 8 is reached. At this stage the rudiments of the suckers are present as lenticular, cellular aggregates (Figs. 8, 22) and the posterior end of the body contains a cavity or a mass of spongy, vesicular tissue. The anterior end of the larva, which contains the sucker rudiments, is then retracted and the sides close over it to form the wall of the cyst. At first the closure is incomplete and slight pressure on the cyst causes the contents to be everted (Fig. 9). When the cyst is formed, the cercomere is longer than the diameter of the cyst and some of the hooks of the onchosphere can usually be found at or near the posterior end of the cercomere. With further development, the scolex of the cestode is differentiated and the suckers acquire a cup-shaped form. The cercomere shrivels, and in the cysticercoids removed from naturally infected mites, it was merely a fibrous appendage to the cyst. There is much evidence to show that the rate of development in the intermediate host is highly variable. Many factors probably contribute to this result; the species of mite, number of larvae present in the haemocoele, relative age and degree of development of particular larvae, etc. This subject was discussed in the paper on the development of *Moniezia* in the intermediate host (Stunkard, 1939a), and the same factors probably operate in both *Moniezia* and *Cittotaenia*. The time required for the cysticercoid to complete its development and become infective is unknown, but probably three or four months is the usual period. The rate is undoubtedly affected by temperature, and development is more rapid during the summer months.

The taenioid cestodes have two larval stages in the life cycle, each infective for the appropriate consecutive host. The onchosphere completes its development in the egg and when released this larva progresses with the hooks in advance. The vermiform larva, which is formed by multiplication of germinal cells present in the onchosphere and which develops into the cysticercoid, moves with the other end forward, i.e., the one opposite the larval hooks. There is, accordingly, a metamorphosis between the two larval stages, a reorganization which involves replacement of the cellular elements, and a reversal of the anteroposterior axis of the larva. The vestiges of the onchosphere together with the hooks are cut off in the degenerating cercomere at the posterior end of the second larva. The scolex, which develops from the anterior end of the second larva, remains the physiological anterior end of the definitive cestode.

It is apparent that rabbits acquire the infection by accidentally eating mites which contain cysticercoids. About eleven weeks are required for the development of *C. ctenoides* in the final host, i.e., from the ingestion

of the cysticercoid until the appearance of eggs in the feces. Pertinent data concerning the similar period in the development of *Andrya cuniculi* were reported by Stunkard (1934). A rabbit taken on April 24th, 1932, and brought to the animal house on April 28th was dissected on May 18th and contained a small, sexually immature specimen which bore the terminal proglottid. Another rabbit caught at the same time began to pass eggs of *A. cuniculi* on July 1st, 67 days after it was captured. Since there was no opportunity for infection in the animal house, the animal must have ingested the parasite at least ten weeks before eggs were passed in the feces.

The development of *C. ctenoides, C. denticulata* and *C. pectinata* in their final hosts has been traced by the removal of young worms from the intestines of naturally infected rabbits and hares. A selected series, illustrating the growth of each species is presented in Plates IV and V. The identification of the worms was made by tracing back successively younger stages from ones which were readily identifiable. Differences in size of scolices and suckers, together with relative development of proglottids and reproductive organs, were used for specific determination. The photographs were made from fixed and stained specimens and the size of each is given in the explanation of the figure.

Evans (1940) referred to reports by various authors who found specimens of *Cittotaenia* in the body cavity of rabbits. He stated, "These continued records of species of *Cittotaenia* being found in the body cavity are interesting, since they support the contention of Stoll (1937) that *Moniezia expansa* migrates in the body of the sheep during the earliest part of its life in the host." In commenting on the suggestion of Stoll, Stunkard (1939a) pointed out that it is doubtful to what extent his data support the hypothesis of a preliminary tissue invasive stage of *M. expansa* in sheep.

Like Evans and others, I have often found specimens of *Cittotaenia* in the body cavity of rabbits and hares that had been shot in the field and examined some time later. An observation by Riehm (1881) has a significant bearing on this subject. Referring to *C. pectinata* he stated, "Sie bewohnen meist den vorderen Abschnitt des Dünndarmes, nahe am Magen, doch darf man es auch nicht unterlassen, in der Leibeshöhle, namentlich zwischen den Lappen der Leber nach ihnen zu suchen, weil sie häufig genug durch die mörderische Schrotkugel aus ihrem eigentlichen Wohnsitze herausgerissen werden oder auch durch eine auf diesem Wege entstandene Öffnung in der Darmwandung nach dem Erkalten ihres Wirtes herauskriechen." My experience agrees with that of Riehm; I have never found a cestode in the body cavity of a rabbit that had not been shot and know of no record of such an occurrence. Observation of abnormal and post-mortem conditions may easily lead to unwarranted

conclusions. After the host has been dead for several hours and degenerative changes have begun in the intestine, the worms migrate through any available opening but, so far as I am aware, specimens of *Cittotaenia* do not pass through the intestinal wall of live and healthy rabbits.

Even if older cestodes normally leave the intestine, which is very unlikely, it does not follow that the very young worms do so. The idea that specimens of *Cittotaenia* undergo an early developmental phase in the tissue or in the body cavity of the rabbit finds no support from the present investigation. The cysticercoid of *C. ctenoides* (Fig. 25) which developed in a domestic rabbit, contained a scolex 0.13 mm in width with suckers 0.06–0.07 mm in diameter. In a young worm of the same species, which when permanently mounted measured 1.1 mm in length (Fig. 34), the scolex was 0.165 mm in width and the suckers 0.069 mm in diameter. Some shrinkage undoubtedly resulted from the fixation and mounting of the young worm, but the small difference in size of scolex and suckers indicates that the specimen had not passed through a developmental stage in the tissue or body cavity of the rabbit. Furthermore, the smallest specimen of *C. denticulata* removed from the intestine (Fig. 26) is only 0.3 mm in length and 0.16 mm in width. It is only slightly larger than the scolex of the cysticercoid. There appears little possibility that it had developed in some extra-intestinal location. Indeed, the finding in the intestines of rabbits, of more than 200 specimens of *Cittotaenia* which were less than 10 mm in length, negates the hypothesis that the young worms have a developmental stage during which they leave the intestine. It is probable that the scolices, after liberation from the cysts, attach firmly to the intestinal wall and between the villi, but no critical evidence has so far been submitted to demonstrate a tissue invasive or peritoneal stage in their development.

ABSTRACT SUMMARY

Thousands of free-living mites were collected near Hamburg from areas where wild rabbits were numerous and heavily infected with anoplocephaline cestodes. The mites were dissected and cysticercoids were removed from *Scutovertex minutus, Scheloribates laevigatus* and *Pelops tardus*. The larvae were fed to young, domestic rabbits and a cysticercoid from *S. minutus* developed into a sexually mature specimen of *Cittotaenia ctenoides*. Other mites, collected from areas where they would not be exposed to eggs of anoplocephaline cestodes, were fed eggs of *Cittotaenia ctenoides* and *C. denticulata*. Developmental stages of *C. ctenoides* were recovered from the body cavities of *Scutovertex minutus, Galumna obvious, Pelops acromius, Liacarus coracinus, Notaspis coleoptratus, Liebstadia similis, Xenillus tegeocranus, Scheloribates laevigatus, Cepheus cepheiformis, Trichoribates incisellus,* and *Galumna*

norvegicus. The larvae attained the cysticercoid stage in the first seven of the species listed and, since development appeared normal in the other mites, it is possible that they might have completed their development if the hosts had lived long enough. Development of *C. denticulata* was observed in *S. minutus*, *X. cheopis*, *C. cephaliformis*, *T. incisellus*, *S. laevigatus*, and *L. echinus*, although only *S. minutus* lived long enough in the cultures for cysticercoids to be produced. Infections were obtained only in oribatid mites. None of the rabbits fed cysticercoids from experimentally infected mites gave evidence of infection and it is probable that these larvae were not entirely mature. The developmental stages of *C. ctenoides* and *C. denticulata* in the intermediate hosts are described from experimental infections. The development of *C. ctenoides*, *C. denticulata* and *C. perrimani* in the final hosts is described from natural infections. No evidence was found to support the idea that these tapeworms migrate from the intestine to the body cavity of normal, live, rabbits.

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Explanation of Plates

Plate I

Unretouched photographs of living precystic stages in the development of *C. cenchides*. All specimens were removed from experimentally infected mites. Details concerning dates of exposure and dissection are presented in the text.

Fig. 1. Onchosphere, 0.025 × 0.028 mm, from *Scutovertex minutus*.

Fig. 2. Spherical larva, 0.095 mm in diameter, from *S. minutus*.

Fig. 3. Spherical larva, 0.11 × 0.1 mm, from *Trichoribates incisellus;* note the cavity and blistered surface.

Fig. 4. Oval larva, 0.2 × 0.16 mm, from *Galumna obvious;* note the large cavity.

Fig. 5. Pyriform larva, 0.36 × 0.26 mm, from *Liacarus coracinus;* note the large germinal cells.

Fig. 6. Vermiform larva, 0.413 × 0.18 mm, from *G. obvious*.

Fig. 7. Larva with beginning of cercomere formation, body 0.3 mm long, from *G. obvious*.

Fig. 8. Pyriform larva with completely formed cercomere, body 0.31 mm long, from *G. obvious*, note the rudimentary sucker pads near the anterior and the loose tissue near the posterior end of the body.

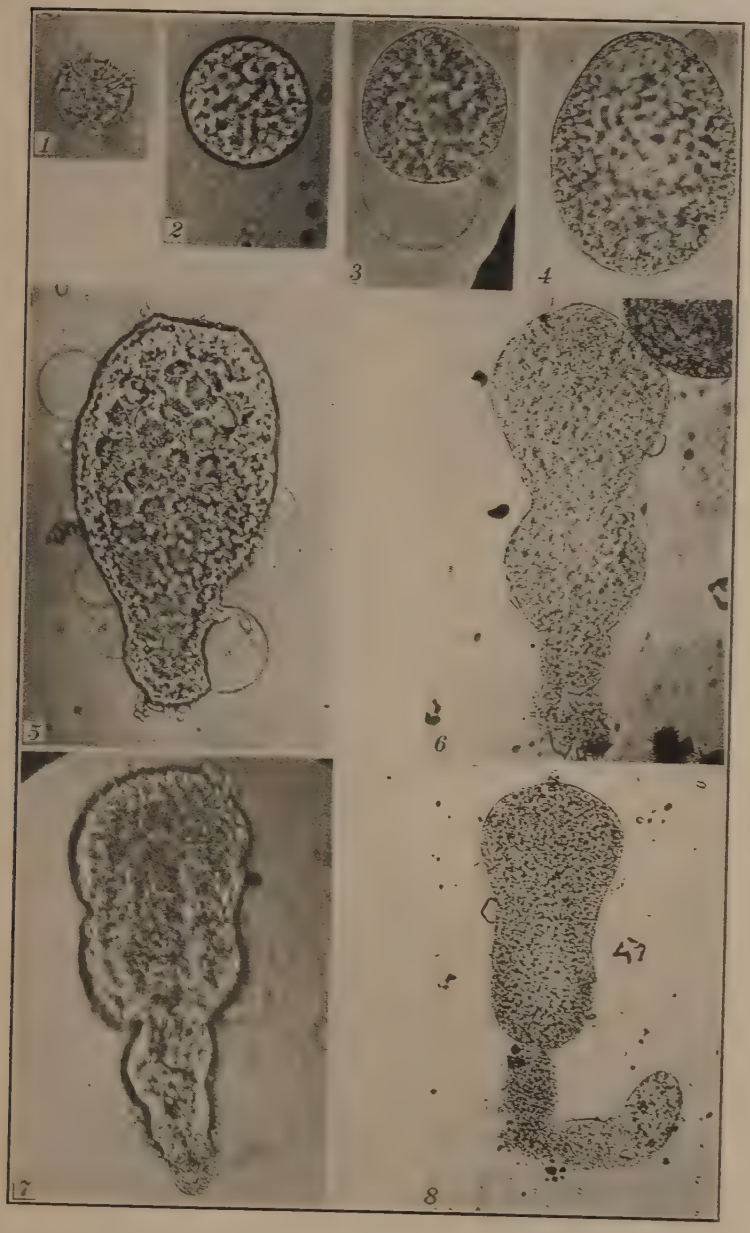
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Plate II

Unretouched photographs of living postcystic stages in the development of *C. ctenoides* and *C. denticulata*. All specimens were removed from experimentally infected mites. Details concerning dates of exposure and dissection are presented in the text.

Fig. 9. *C. ctenoides*, young cysticercoid from *Pelops acromius*, cyst 0.2×0.18 mm; contents of cyst extruded on the application of a coverglass.

Fig. 10. *C. denticulata*, cysticercoid from *Scutovertex minutus*, cyst 0.15×0.13 mm; note the coiled cercomere.

Fig. 11. *C. ctenoides*, eleven cysticercoids, all from a single specimen of *Xenillus tegeocranus*, largest cyst 0.18×0.15 mm.

Fig. 12. *C. ctenoides*, two cysticercoids and one vermiform larva from *Galumna obvious*. The vermiform larva is shown more highly magnified in Fig. 6. The cysticercoid adjacent to the vermiform larva is shown in Fig. 13, the other in Fig. 14.

Fig. 13. *C. ctenoides*, cysticercoid, 0.17×0.17 mm; evaporation of water and pressure of the coverglass has caused the emergence of a little material at the apex of the cyst.

Fig. 14. *C. ctenoides*, cysticercoid, 0.2×0.19 mm.

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Plate III

Free-hand drawings of eggs and larvae of *C. ctenoides* and *C. denticulata.* The rate of development is variable and the ages given are estimated.

Fig. 15. *C. ctenoides,* egg with hyaline shell, from an attached proglottid.

Fig. 16. *C. denticulata,* egg from a moist chamber, several days after removal from a detached proglottid.

Fig. 17. *C. ctenoides,* onchosphere from body cavity of a mite, 1–2 weeks development.

Fig. 18. *C. ctenoides,* spherical larva, 2–3 weeks development.

Fig. 19. *C. ctenoides,* spherical larva, 4–5 weeks development.

Fig. 20. *C. ctenoides,* pyriform larva, 6–8 weeks development.

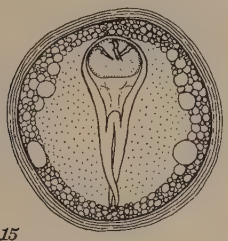
Fig. 21. *C. ctenoides,* vermiform larva, 8–10 weeks development.

Fig. 22. *C. ctenoides,* pyriform larva with cercomere, 10–12 weeks development; drawing based on specimen shown in Fig. 8.

Fig. 23. *C. ctenoides,* young cysticercoid with long cercomere, 10–12 weeks development.

Fig. 24. *C. denticulata,* cysticercoid with cercomere; drawing based on sketches and photograph of specimen shown in Fig. 10.

Fig. 25. *C. ctenoides,* cysticercoid, drawn from pencil sketches of specimen, removed from a naturally infected *Scutovertex minutus,* which developed into a sexually mature cestode in the intestine of a domestic rabbit.



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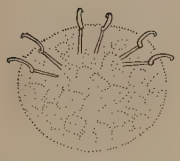

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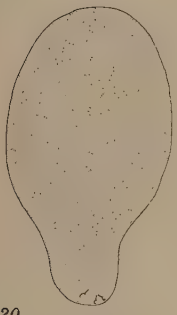

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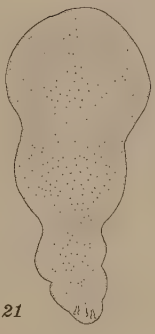

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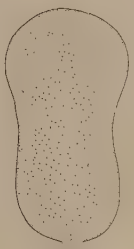

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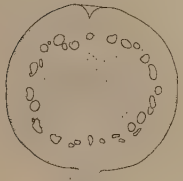

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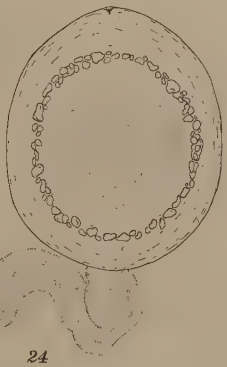

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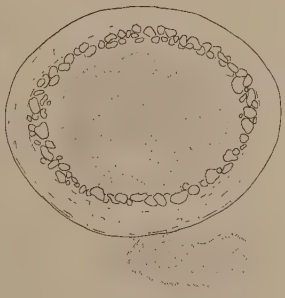

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PLATE IV

Unretouched photographs of young stages of *C. denticulata*, made from fixed and stained preparations. Actual size of the specimens is given, in millimeters.

	Length of specimen	Diameter of scolex	Size of suckers
FIG. 26.	0.3	0.16	0.07 ×0.055
FIG. 27.	0.414	0.21	0.108×0.08
FIG. 28.	0.885	0.26	0.13 ×0.1
FIG. 29.	1.46	0.288	0.14 ×0.11
FIG. 30.	3.2	0.38	0.18 ×0.16
FIG. 31.	5.5	0.46	0.216×0.18
FIG. 32.	8.3	0.5	0.24 ×0.2
FIG. 33.	12.	0.54	0.25 ×0.24

Under size of suckers, the larger measurement is the diameter and the smaller one is the depth of the sucker. In mature specimens the scolex measures 0.5 to 0.9 mm and the suckers 0.2 to 0.3 mm. The scolex, therefore, attains its growth early.

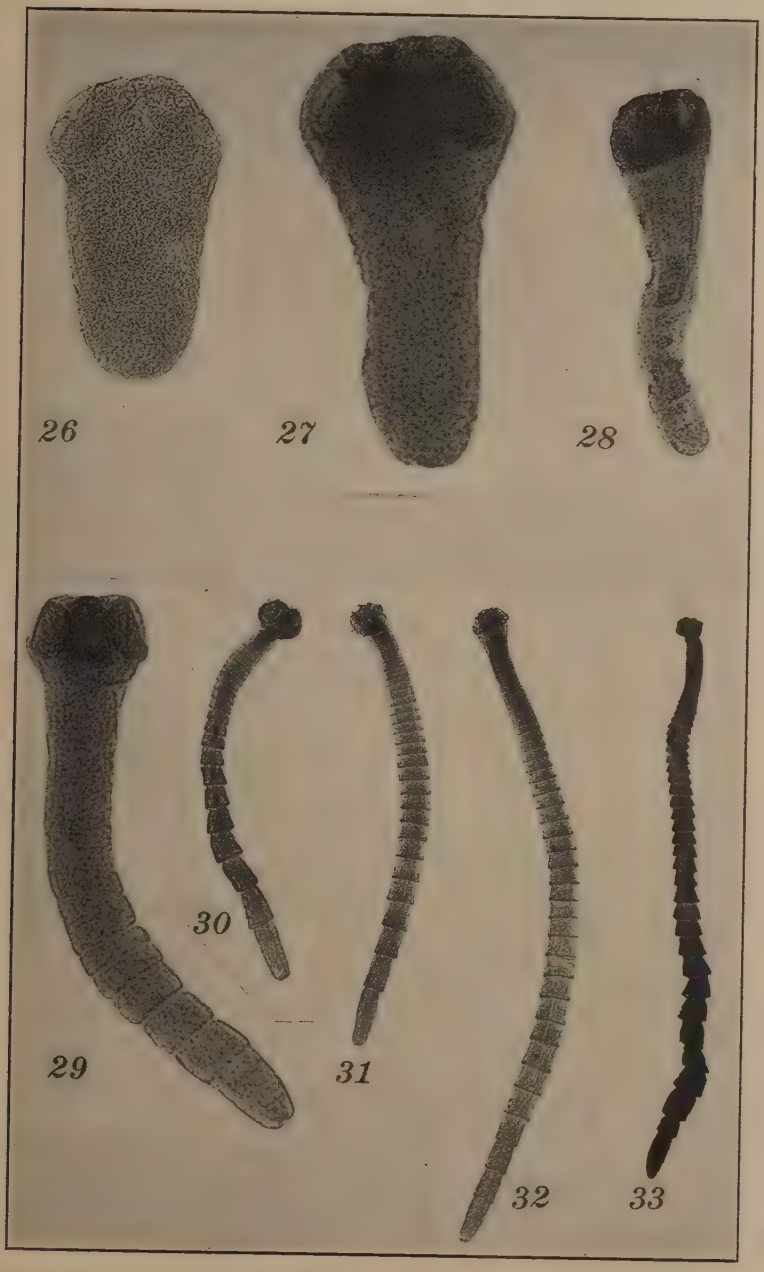
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Plate V

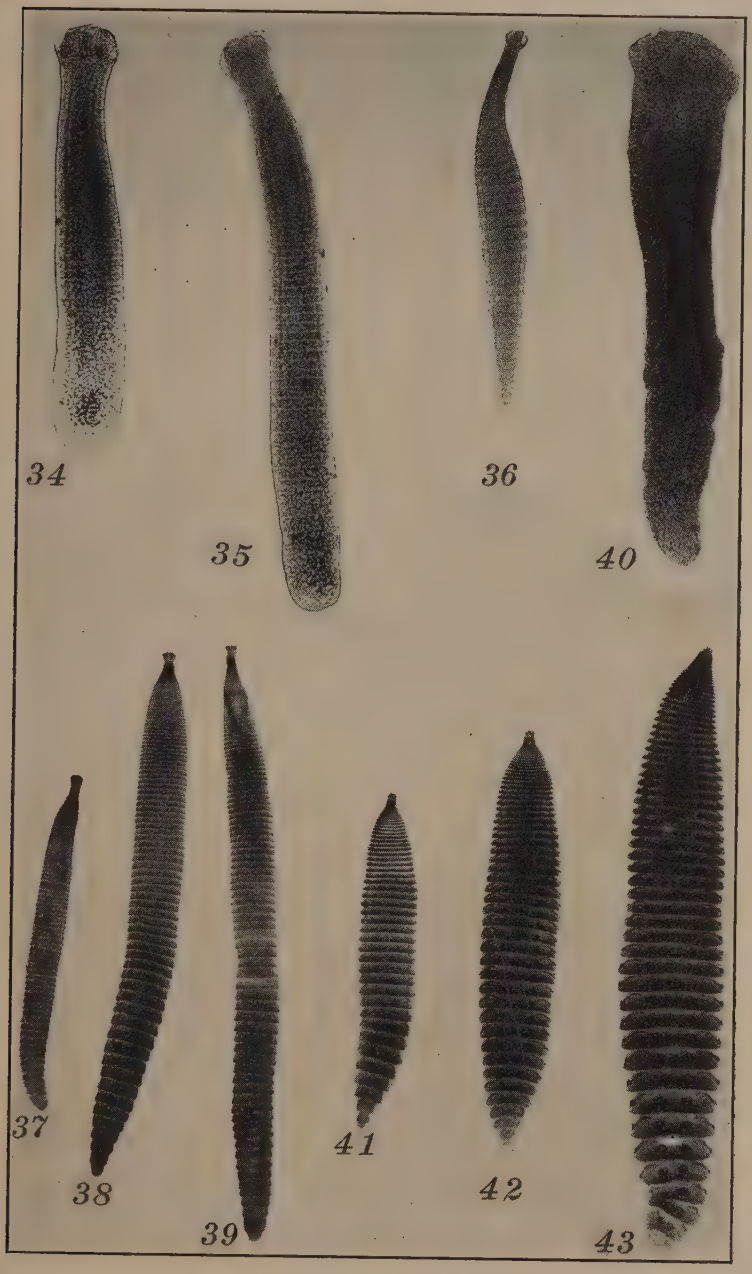
Unretouched photographs of young stages of *C. ctenoides* and *C. pectinata*, made from fixed and stained preparations. Actual size of the specimens is given, in millimeters.

	Length of specimen	Diameter of scolex	Size of suckers
Fig. 34. *C. ctenoides*	1.1	0.165	0.069 × 0.055
Fig. 35. *C. ctenoides*	1.9	0.2	0.09 × 0.072
Fig. 36. *C. ctenoides*	4.6	0.28	0.144 × 0.12
Fig. 37. *C. ctenoides*	8.2	0.316	0.158 × 0.14
Fig. 38. *C. ctenoides*	13.2	0.36	0.16 × 0.16
Fig. 39. *C. ctenoides*	15.	0.38	0.166 × 0.16

In mature specimens the scolex measures 0.35 to 0.5 mm in diameter and the suckers 0.15 to 0.24 mm.

Fig. 40. *C. pectinata*	0.97	0.2 (flattened)	0.075 × 0.055
Fig. 41. *C. pectinata*	9.5	0.25	0.128 × 0.11
Fig. 42. *C. pectinata*	11.8	0.28	0.14 × 0.14
Fig. 43. *C. pectinata*	17.	0.28	0.14 × 0.14

The scolex in Fig. 42 has reached the normal size and little if any growth is made later.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STUDIES ON THE MORPHOLOGY OF THE *E. HISTOLYTICA*-LIKE AMOEBAE FOUND IN MONKEYS

HERBERT SALIS*
Department of Zoology, University of Pennsylvania

With the advent of the present century, protozoölogists began to evidence interest in the protozoa to be found in primates other than man. In the steady accumulation of literature, the amoebic organisms found in monkeys have received much attention, mainly because of their apparently close relationship to the endamoebae in human hosts. Much has already been published in attempts to demonstrate the similarity or dissimilarity between the intestinal endamoebae in man and those in monkeys, which two hosts are, in themselves, of close evolutionary relationship.

Investigators who first encountered amoebae in monkeys often erected new species names without adequate distinction from those already known in man. Since then, however, there has evolved the increasingly accepted belief that the endamoebae of man and monkey are identical, and recent literature divides the endamoebae of monkeys into those similar, or identical, to *Endamoeba coli* in man, and those similar, or identical, to *Endamoeba histolytica* in man.

This paper will attempt to establish that the amoebae of monkeys, with specific reference to the *E. histolytica*-like type, are *not* all identical with those in man. The writer has employed both qualitative and quantitative tests of more precise nature than heretofore employed, which, it is believed, will reveal more clearly the morphological differences among the *E. histolytica*-like amoebae parasitic in monkeys.

MATERIALS AND METHODS

Prepared slides containing *Endamoeba histolytica* from six human hosts, as well as the slides from the monkeys, were furnished by Dr. Wenrich. *E. histolytica*-like amoebae were studied from fecal smears of twelve different kinds of monkeys, involving seventeen individual hosts, whose names and sources are given in Table 3. Since the nomenclature of apes and monkeys is still in a highly confused state, the system adopted by Stiles and Nolan (1929) has been employed.

Slides fixed in Schaudinn's fluid, including five per cent of acetic acid, and stained with Heidenhain's haemotoxylin, with iron alum dif-

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ferentiation, have been mainly used A few slides from the monkeys had been prepared by other methods.

The mensurative procedures are similar to those used by Dobell and Jepps (1918) in their study upon size races of *Endamoeba histolytica* cysts from man. As these investigators found, few of the many cysts measured were perfectly circular in profile. Therefore, most of the recorded dimensions of cysts were the mean of the shorter and longer diameters of subcircular organisms. Cysts markedly deformed in shape, infrequently seen, have not been included. In reality, cyst measurements were of the stained cytoplasmic contents within the cyst wall. As Dobell and Jepps have pointed out, the balsam mounting renders the cyst wall too indistinct for inclusion in the measurement of the cyst. Therefore, measurements do not include the cyst wall which is approximately half a micron thick, as noted by the above authors.

Although the physical handicaps in measurements have been considerable, all dimensions of nuclei have been ascertained with the greatest possible care in each case. The mean diameter of nuclei in material fixed by Yocum's picromercuric was found to be slightly greater than in material fixed with Schaudinn's fluid, but these dimensions were not used. Degenerate and obviously hypertrophied nuclei have not been measured, but these were very infrequent among the many observed.

Observations were made with a 10× ocular and a 2 mm, 1.30 N A oil immersion objective. For measurements a calibrated ocular micrometer, with a value of 0.94 μ for each subdivision, was utilized.

Through a conversion table, measurements were converted directly into micron values. Where the diameter of the cyst or nucleus did not lend itself to definitive measurement, the measurement was made to the nearest micrometer unit. By this method, the maximum personal observational error was kept as close as possible to plus or minus half a micron, a spread of one micron or one micrometer division. This agrees with the findings of Dobell and Jepps, which demonstrated that, with very careful measurement, "the individual error would not be greater than one unit of the scale employed."

Endamoeba histolytica Schaudinn

This species was found upon slides from some of the monkeys studied (Table 3). The trophozoites present possessed the typical appearance of *Endamoeba histolytica* from man. The two large lobular pseudopodia shown in Fig. 5 are suggestive of active movement. Food bodies of various sorts were included in the organism. The rounded nucleus appeared to be more delicate than cyst nuclei. Precystic stages (Fig. 1) were highly vacuolated, and possessed a small nucleus, showing only peripheral chromatin, a central karyosome, and several radii.

The chromatoids in the cysts were very much like those in cysts of *E. histolytica* from man. Although variations occurred, they characteristically were large, usually regular in outline, bar-like or fusiform in shape, and few in number. The glycogen vacuole, although infrequent, was clear and usually large.

Table I shows that the percentages in regard to nuclear number of *E. histolytica* cysts from monkey material compare well with those of

TABLE 1.—*Nuclear number in cysts of* E. histolytica *from monkey and man*

No. of nuclei	100 cysts from			
	Monkey	*Homo* No. 1	*Homo* No. 2	*Homo* No. 3
1-nucleate	15	19	7	44
2-nucleate	25	24	20	12
3-nucleate	4	2	4	3
4-nucleate	56	55	66	41
8-nucleate	..	..	3	..

cysts of the species from human hosts. Even with a large number of trophozoites present, thus reducing the possibility of mature cysts, the number of quadrinucleate cysts from human host No. 3 is still close to half the sum of tabulated cysts.

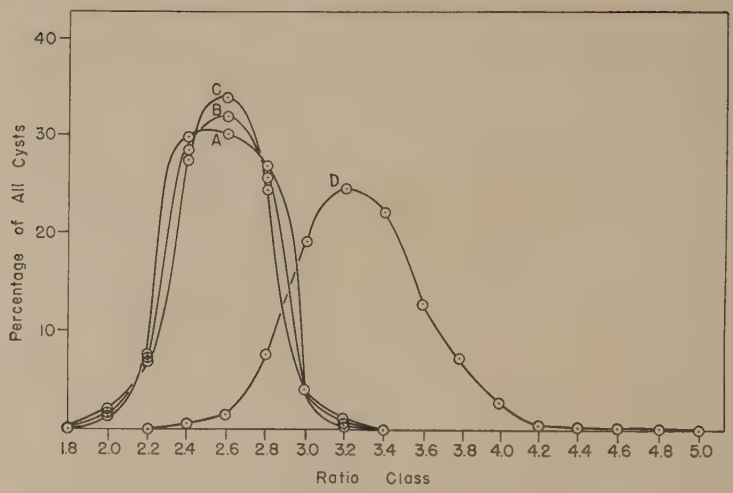
A study was made of the size relationship between the uninucleate cyst and its nucleus, expressed as the ratio of the mean diameter of the cyst to the mean diameter of the contained nucleus. Cysts possessing nuclei in mitotic stages were excluded because of their disturbing effect upon the general cyst-nucleus ratio. The results of this mensurative analysis, and their comparison with those obtained, in the same way, from uninucleate cysts of *E. histolytica* from man are shown in Table 2 and Graph I.

If the value of t is less than 2, then the difference between the means of different samples is not likely to be significant. Likewise, if the value of t exceeds 2, the difference is probably significant. Davenport and Ekas (1936) state that, "Of course, the more the difference between means exceeds the limit of $2\times$ the standard error of the means, the more certainly significant the difference becomes." Since t in Table 2 is less than 1, the difference in the means of the samples from man and from monkey is certainly not significant.

Two size races of the *E. histolytica* organisms in the monkey have been determined. In the measurements, only uninucleate cysts were involved. From one host, a race with a mean of 7.9 μ was discerned with the following range:

Diam. in μ	6.0	7.0	8.0	9.0	10.0
No. of cysts	1	4	22	7	1 = 35

This size race is comparable in size and appearance to the small race of *E. histolytica* in man, commonly called *E. hartmanni.*



GRAPH I. Cyst-nucleus ratios: A—*E. histolytica* (man); B—*E. histolytica* (man and monkey); C—*E. histolytica* (monkey); D—*E. chattoni.*

The other size race possessed a mean of 11.28 μ and the following range:

Diam. in μ	8.0	9.0	10.0	11.0	12.0	13.0	14.0
No. of cysts	1	12	25	68	54	17	1 = 178

The cytoplasmic parasite, *Sphaerita* sp., has been observed in one cyst (Fig. 10).

This species was of small numbers in the material from the monkey. Since the number of hosts and the amount of material have been quite extensive, this condition suggests that this species of *Endamoeba* is not of extensive occurrence in the natural state of monkeys in general.

TABLE 2.—*Cyst-nucleus ratios of* E. histolytica *in man and monkey*

Ratio class	No. of cysts in class: Man	Monkey	Combined series
1.90–2.09	3	4	7
2.10–2.29	19	14	33
2.30–2.49	75	55	130
2.50–2.69	77	68	145
2.70–2.89	68	49	117
2.90–3.09	10	8	18
3.10–3.29	1	2	3
N =	253	200	453
*Mean =	2.57	2.59	2.58
$^*\sigma$ =	0.198	0.215	0.210
S. E. =	± 0.012	± 0.015	± 0.010

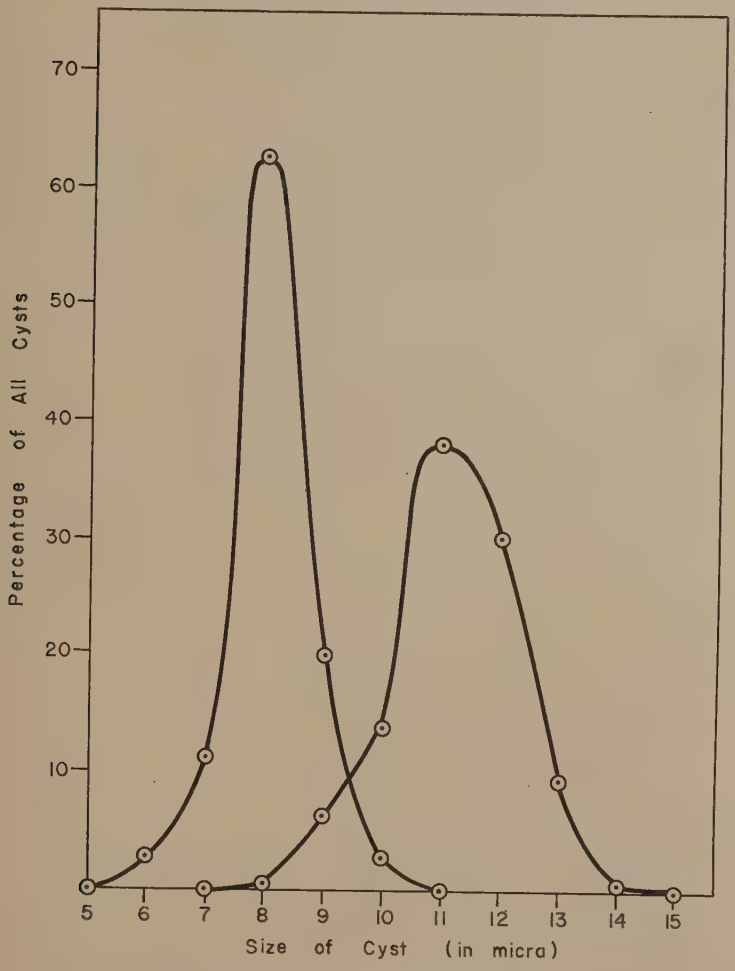
$$t = 0.964 = \frac{\text{difference between means}}{\text{standard error of difference between means}} = \frac{\text{Mean}_B - \text{Mean}_A}{\sqrt{\text{S. E.}^2_B + \text{S. E.}^2_A}}$$

* The method used in calculating the mean and its standard deviation was the "short-cut" procedure explained in Hegner and Andrews (1927).

Endamoeba chattoni Swellengrebel

This uninucleate species is much more characteristic of the monkey than is *Endamoeba histolytica*. *E. chattoni* not only was found in more hosts (Table 3), but was also far more numerous wherever it was disclosed. If the material utilized here is representative of monkeys in general, then these uninucleate cysts are to be considered as typical of monkeys in general.

Representative trophozoites (Fig. 11), of the few encountered, dis-



GRAPH II. Size races of *E. histolytica* (in monkey).

played a somewhat vacuolated endoplasm containing food bodies, apparently bacteria. Where ectoplasm was to be distinguished, the pseudopodia were broad, and only slightly extended. The nucleus was of the *E. histolytica* type, and presented a regular round or oval shape.

The uninucleate nature of *Endamoeba chattoni* was reaffirmed. No cysts above a binucleate stage were observed. Of 850 cysts examined in a study, only 8 binucleate cysts were seen. Thus, the uninucleate cysts definitely constitute the mature stage of development, and the binucleate cysts compose a supernucleate stage of an average incidence of less than one per cent. The binucleate cysts are commonly larger than the uninucleate, just as supernucleate cysts are usually larger among other endamoebae.

The size relationships between the cyst and the nucleus in the uninucleate cysts have been studied in the same way as those of *E. histolytica*. The data are presented in Table 4 and Graph I. The mean of the cyst-nucleus ratios of the *E. histolytica* cysts from man, the mean of those of the same species in the monkey, and the mean of the combined

TABLE 3.—*Incidence of* E. histolytica-*like amoebae in monkeys studied*

Monkey host	Species of *Endamoeba*		Source of host
	histolytica	*chattoni*	
Anubis baboon (*Papio anubis*)	−	+	Phila. Zool. Garden
Gelada baboon (*Theropithecus gelada*)	+	+	" " "
Guinea baboon (young) (*Papio papio*)	+	−	W. Buck's Menagerie
Mandrill (young) (*P. sphinx*)	−	+	" " "
Mandrill (*P. sphinx*)	+	+	Phila. Zool. Garden
Dwarf monkey (*Cercopithecus talapoin*)	+	+	" " "
Green monkey (*C. callitrichus*)	+	+	" " "
Japanese macaque (*Silenus fuscatus*)	+	−	" " "
Kra monkey (*S. irus*)	−	+	" " "
Kra monkey (*S. irus*)	−	+	" " "
Mangaby monkey (*Cercocebus* sp.)	−	+	" " "
White-crowned mangaby monkey (*C. aethiops*)	+	+	" " "
Mona monkey (*Cercopithecus mona*)	−	−	W. Buck's Menagerie
Mona monkey (*C. mona*)	+	+	" " "
Mona monkey (*C. mona*)	−	+	" " "
White-nosed monkey (*C. fantiensis*)	−	+	Phila. Zool. Garden
Rhesus monkey (*Silenus rhesus*)	+	+	" " "

ratios of these two groups is in each case of very great significance, since the values of *t* are far above 2.

Although the nucleus is of the *E. histolytica* type, there is an apparent distinction in that the karyosome is smaller, the chromatin granules between the karyosome and the peripheral chromatic granules are finer, and the perikaryosomal ring of granules is less constricted about the karyosome. The peripheral chromatin also appears less dense. The nucleus is thus of more delicate nature, just as that of *E. histolytica* is more delicate than that of *E. coli*. The amount of chromatin, especially karyosomal, is, therefore, in direct proportion to the number of nuclear divisions to be undergone in the cyst.

TABLE 4.—*Cyst-nucleus ratios of* E. chattoni

Ratio class	No. of cysts in class
2.30–2.49	3
2.50–2.69	8
2.70–2.89	43
2.90–3.09	107
3.10–3.29	138
3.30–3.49	123
3.50–3.69	72
3.70–3.89	41
3.90–4.09	16
4.10–4.29	3
4.30–4.49	1
4.50–4.69	1
4.70–4.89	1
N =	557 + 453 = 1010 = Total (*E. chattoni* + *E. histolytica* (man and monkey))

Mean = 3.29
σ = 0.327
S. E. = ± 0.014

E. chattoni vs. *E. histolytica* (man), t = 38.7
E. chattoni vs. *E. histolytica* (monkey), t = 33.4
E. chattoni vs. *E. histolytica* (man and monkey), t = 41.8

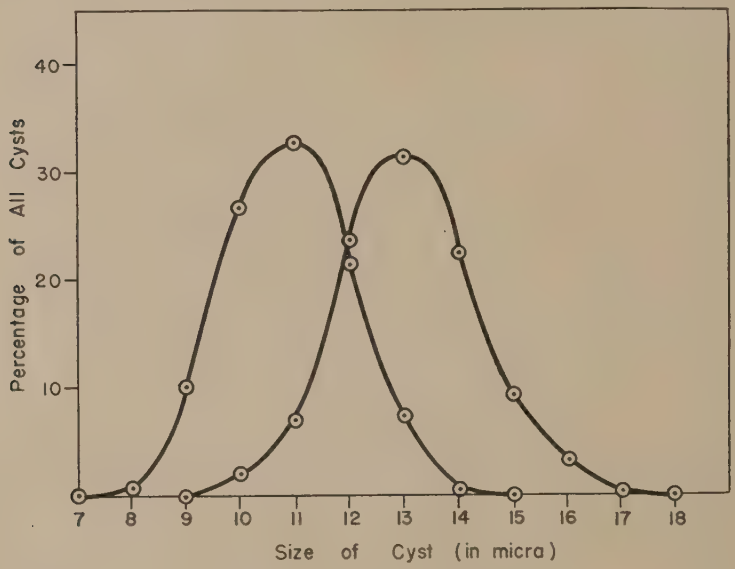
Large numbers, small size, and irregularity in shape characterize the chromatoids in the cysts. The usual chromatoids are small and rounded or irregularly angular (Fig. 15). At times, small granular chromatoids, as many as 250 in a single cyst, would obscure or hide the nucleus. To estimate the number in cysts containing masses of minute particles is almost impossible. The lower numerical limits are about 15–20 chromatoids, and the average number may be set conservatively at 40–60 chromatoids. The larger cysts do not appear to contain more chromatoids despite a maintained smallness in chromatoid size (Fig. 13).

A strain of *E. chattoni,* seen in three hosts, presented less numerous chromatoids, some of which were large in size and regular in shape. Most frequent were cysts containing 15–20 chromatoids of highly diversified shape and size. A series of chromatoidal conditions could be distinguished in which the size decreased and the shape became more irregular with increase in number. This began with a cyst containing two large and regular bar-shaped chromatoids (Fig. 17), and concluded with one having approximately sixty-two chromatoids of characteristic types (Fig. 18). Paired chromatoids of the more regularly contoured kind were frequent throughout the series.

Commonly found within the cysts was a small, dense, homogeneously stained area, of a light greyish brown color. This round or oval area has been interpreted as a glycogen vacuole, possibly a remnant. It is small in comparison with that found in *E. histolytica.*

Vacuoles of puzzling nature were found in a few cysts, usually occupying roughly half the cyst diameter. The vacuole, containing the nucleus, would be outlined generally by the small chromatoids lying around it, a characteristic of the glycogen vacuole in the binucleate cystic stages of *Endamoeba coli.* Fig. 14, however, shows chromatoids both

inside and outside the vacuole, whose boundary is clearly seen. The area within the vacuole was clearer than the circumjacent cytoplasm, suggesting a former glycogen content later dissolved out. But, despite the similarity to the binucleate stage in *E. coli,* the location of the nucleus and even chromatoids within the vacuole renders this consideration improbable.



GRAPH III. Size races of *E. chattoni.*

Size races were not easily established, but two races were measured. The smaller race was obtained from four hosts and furnished the following data:

Diam. in μ	8.0	9.0	10.0	11.0	12.0	13.0	14.0	
No. of cysts	3	38	104	127	83	29	2	= 386

Mean = 10.93 μ
σ = 1.051
S. E. = ± 0.053

The larger race, from three hosts, was recognized by the following measurements and data:

Diam. in μ	10.0	11.0	12.0	13.0	14.0	15.0	16.0	17.0	
No. of cysts	8	28	95	127	90	38	13	1	= 400

Mean = 13.06 μ
σ = 1.178
S. E. = ± 0.059

The means of these two size races are of a definitely significant difference, since in their comparison the value of t is 26.7, far above 2.

DISCUSSION

Observation of stained preparations of the so-called *E. histolytica*-like amoebae in monkeys has established by the comparison in the cysts of chromatoids, glycogen areas, nuclear number in developmental stages, cyst-nucleus size relationship, and nuclear structures, that there can be differentiated two species of amoebae. This is also somewhat corroborated by the restricted study of the trophozoite stages.

Since the more recent literature largely expresses the opinion that the *E. histolytica*-like amoebae in monkeys are very similar to, if not identical with *Endamoeba histolytica* in man, an important result of the studies reported in this paper is the definite identification of *E. chattoni*.

Chatton (1912) was observing cysts of *E. chattoni* among his "trophozoites" of "*Löschia* sp." from *Macacus sinicus*. Not only are there no polynucleates, but the measurement of the cysts and nuclei in his five drawn uninucleate "trophozoites" disclosed a mean ratio of 3.3 and a range of 3.1 to 3.6

Swellengrebel (1914) observed in *Macacus rhesus* the organisms described by Chatton, and believed them to be a new species, *Entamoeba chattoni*. Aside from a few binucleate cysts, no polynucleate cysts were found. He described the trophozoites as alveolar, mostly free of inclusions, and with very little ectoplasm. Of five drawn uninucleate cysts, the mean cyst-nucleus ratio was found to be 3.2, with a range of 2.9 to 3.6.

Behrend (1914), aside from his seeming confusion of *E. histolytica*- and *E. coli*-like cysts, appears to have seen both *E. histolytica* and *E. chattoni* in material from *Macacus rhesus*. The former species is indicated by the description of quadrinucleate cysts, often with single large chromatoids. But uninucleate cysts with paired thin chromatoids, and the rarity of polynucleate cysts indicate the probable presence of *E. chattoni* cysts.

In this period, Castellani (1908) had erected the first new species, *Entamoeba nuttali,* based upon trophozoites of vague morphology from *Macacus pileatus,* and Mathis (1913) had proposed the new species, *Löschia duboscqi,* apparently for organisms of *Endamoeba histolytica* from *Macacus rhesus* and *M. tchliensis*.

Bach (1923) and Mello (1928) studied similar trophozoites and cysts from *Macacus rhesus*. Bach presents five drawings of uninucleate cysts whose cyst and nucleus measurements furnished a mean ratio of 2.6, with a range of 2.4 to 2.9. Since groups of ten cysts were found sufficient to approximate the mean ratio, there is no doubt that Bach was considering cysts of *E. histolytica*. There remains the possibility of his ignoring *E. chattoni,* since he mentions occasional cysts filled with "splintered and short rod-shaped chromotoids," and discloses that most

of the cysts were uninucleate. In addition, most of the trophozoites possess small, bluntly lobular pseudopodia, and are 13–15 μ in size. These resemble the one depicted in Fig. 11. Thus Bach probably confused the two species and drew cysts of only one.

Hegner and Chu (1930) definitely were studying cysts of *E. chattoni* from wild monkeys, *Macacus philippinensis*. They refer to their necessity to rely on uninucleate cysts, which "contained many paired chromatoidal bodies of various sizes." No polynucleate cysts are mentioned, but a binucleate is drawn together with a uninucleate cyst, whose cyst-nucleus ratio is 3.32. The trophozoite shown is similar to the one (Fig. 11) in this paper. From mean cyst and nucleus diameters of 100 cysts a cyst-nucleus ratio of 2.91 is found. What factors bring the ratio below 3.3 cannot be gleaned from the report.

On the other hand, amoebae maturing with quadrinucleate cysts and morphologically the same as *E. histolytica* from man have been found in *Macacus rhesus* and *M. sinicus* by Dobell and Laidlow (1926) and Dobell and Bishop (1929), to which hosts Dobell (1928, 1931) also added *M. nemestrinus;* in a chimpanzee by Hegner and Schumaker (1928); and in *M. irus, M. rhesus, M. sancti-johannis,* and *M. lasiotis* by Kessel (1928). A more extensive study by Mackinnon and Dibb (1938) established as hosts of these amoebae "a Gorilla, four Chimpanzees, two Gibbons (*Hylobates lar* and *H. lar lenciscus*), a Guinea Baboon (*Papio papio*), a White-crowned Mangaby (*Cercocebus aethiops*), a Sooty Mangaby (*C. fuliginosus*), a Mona Monkey (*Cercopithecus mona*), a Schmidts' Monkey (*C. ascanius schmidti*) and a Patas Monkey (*Erythrocebus patas*)."

The organisms of *E. chattoni* encountered in the literature belong, apparently, almost entirely to the strain described in this paper as producing less numerous and more regularly shaped chromatoids. Drawings depict no more than 25 chromatoids, most of which are of regular shape.

The definite establishment of the uninucleate species in the monkey adds another to the list of such species with *Endamoeba* nuclei. These species have been found in domestic cattle, the horse, the Chinese sheep, the goat, the gnu and in swine.

A brief study of cysts of a species with uninucleate cysts, believed to be *Endamoeba bovis,* in cattle has been made by the author from slides furnished by Dr. Wenrich. Measurement of cyst and nuclear diameters in 20 cysts provided a ratio of 3.22. Chromatoids were typically few in number, but were irregular in shape and of varying sizes. The glycogen vacuoles were clear. The fundamental cyst-nucleus ratio and the irregularity of the chromatoids definitely link this species to that in the monkey. A like similarity of the other species remains to be investigated.

The reports of amoebic dysentery in monkeys naturally raises the question as to which species is the causative agent. From the descriptions, amoebae of the *Endamoeba histolytica* type were responsible. This would be analogous to the situation in man, where *E. histolytica* is considered a primary factor, if not the causative agent, in amoebic dysentery. In addition, the low incidence of this disease in the monkey, coupled with the heavy amoebic infections found among these animals, may be explained in terms of the scarceness of *E. histolytica* in relation to *E. chattoni* in these hosts. By analogy with the other uninucleate species mentioned, which do not appear to be detrimental to their various hosts, it may be expected that the non-pathogenicity of *E. chattoni* will be confirmed.

Research has already been carried out upon the pathogenic role of *Endamoeba histolytica* in the monkey. Among others, Kessel (1928) and Dobell (1931) have performed transfers of amoebae of this type from monkeys to kittens, in which amoebic dysentery has been produced by the organisms. The clinical and pathological evidence from both monkeys and kittens also supports the identity of these amoebae with *E. histolytica* in man. Because of this, the effects of chemical treatment on the pathogenic organism in the monkey have been investigated. Emetine, as in man, has been found to be effective by Dobell and Bishop (1929), who state, "it is suggested that macaques can therefore be utilized . . . in place of men in future chemotherapeutic experiments directed towards the discovery of remedies for human amoebic dysentery." Such experiments, however, must be performed only on monkeys parasitized by *E. histolytica*. The confusion of *E. chattoni* with the dysentery amoeba, as may have occurred in the past, would render invalid the results of such experimentation.

SUMMARY

1. The morphology of the *E. histolytica*-like amoebae in monkeys has been studied in prepared material from twelve different species of monkeys, including seventeen individual hosts.

2. By the use of several differential methods, both *Endamoeba histolytica* and *Endamoeba chattoni* have been identified.

3. The cysts of *E. histolytica* present a cyst-nucleus diameter ratio of 2.6, and two size races with means of 7.9 μ and 11.3 μ were recognized. The organisms are identical with those of *E. histolytica* in man.

4. *E. chattoni* Swellengrebel is validated. It is uninucleate, with a binucleate supernuclear stage of less than one per cent incidence, has a cyst-nucleus diameter ratio of 3.3, and includes at least two size races with means of approximately 10.9 μ and 13.1 μ. Two strains are discernible through their characteristic chromatoids.

5. These uninucleate organisms are believed to be related closely to uninucleate species of similar nature that have been recognized in other animals.

6. Available evidence indicates that *E. chattoni* is non-pathogenic in the monkey.

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Explanation of Plates

Plate I

All figures are of organisms fixed with Schaudinn's fluid, containing 5% of glacial acetic acid, and stained by the iron-haemotoxylin method. Figs. 2, 3, 4 are from the same host, and likewise for Figs. 6, 7, 9, 10. Dimensions are expressed in mean diameters. Magnification 2800×.

Endamoeba histolytica
(in monkey)

Fig. 1. Precystic form of smaller race in a young guinea baboon (*Papio papio*). Size 7.05 μ.

Fig. 2. Average-sized uninucleate cyst of smaller race in a mona monkey (*Cercopithecus mona*). Size 8.0 μ. Nuclear size 3.3 μ. Cyst-nucleus ratio 2.42.

Fig. 3. Binucleate cyst of smaller race in a mona monkey. Remains of a large chromatoid. Size 8.5 μ.

Fig. 4. Quadrinucleate cyst of smaller race in a mona monkey. Size 8.0 μ.

Fig. 5. Elongate trophozoite in a white-crowned mangaby monkey (*Cercocebus aethiops*). Size 23.5 μ × 11.3 μ.

Fig. 6. Average-sized uninucleate cyst of larger race in a mona monkey. Size 10.85 μ. Nuclear size 4.25 μ. Cyst-nucleus ratio 2.55.

Fig. 7. Binucleate cyst of larger race in a mona monkey. Size 12.2 μ.

Fig. 8. Trinucleate cyst of larger race in a gelada baboon (*Theropithecus gelada*). Size 10.35 μ.

Fig. 9. Quadrinucleate cyst of larger race in a mona monkey. Size 12.2 μ.

Fig. 10. Uninucleate cyst of larger race in a mona monkey. Parasitized by *Sphaerita* sp. Size 12.2 μ. Nuclear size 5.65 μ. Cyst-nucleus ratio 2.16.

Plate II

All figures are of organisms prepared as in Plate I. Figs. 13, 15 are from the same host, likewise for Figs. 12, 16, and also for Figs. 17, 18, 19. Dimensions are expressed in mean diameters. Magnification 2800×.

Endamoeba chattoni

Fig. 11. Trophozoite stage in a mangaby monkey (*Cercocebus* sp.). Size 16 μ.

Fig. 12. Average-sized uninucleate cyst of smaller race in an anubis baboon (*Papio anubis*). Small glycogen vacuole remnant. Size 11.3 μ. Nuclear size 3.8 μ. Cyst-nucleus ratio 2.97.

Fig. 13. Average-sized uninucleate cyst of larger race in a kra monkey (*Silenus irus*). Size 13.2 μ. Nuclear size 3.8 μ. Cyst-nucleus ratio 3.47.

Fig. 14. Uninucleate cyst of larger race in a kra monkey. Large vacuole with definite boundary, containing nucleus and some chromatoids. Size 14.6 μ. Nuclear size 4.25 μ. Cyst-nucleus ratio 3.43.

Fig. 15. Uninucleate cyst of smaller race in a mona monkey (*Cercopithecus mona*). Large number of small chromatoids. Size 12.2 μ. Nuclear size 3.8 μ. Cyst-nucleus ratio 3.21.

Fig. 16. Binucleate stage in an anubis baboon. Size 16.45 μ.

Figs. 17–19 represent the strain with fewer, more regular chromatoids.

Fig. 17. Uninucleate cyst in a gelada baboon (*Theropithecus gelada*). Two massive and regular chromatoids, and remnant of glycogen vacuole. Size 12.2 μ. Nuclear size 3.8 μ. Cyst-nucleus ratio 3.21.

Fig. 18. Uninucleate cyst in a gelada baboon. Many varying chromatoids. Size 12.7 μ. Nuclear size 3.8 μ. Cyst-nucleus ratio 3.37.

Fig. 19. Binucleate cyst in a gelada baboon. Chromatoids of more typical number and appearance. Size 16.9 μ.

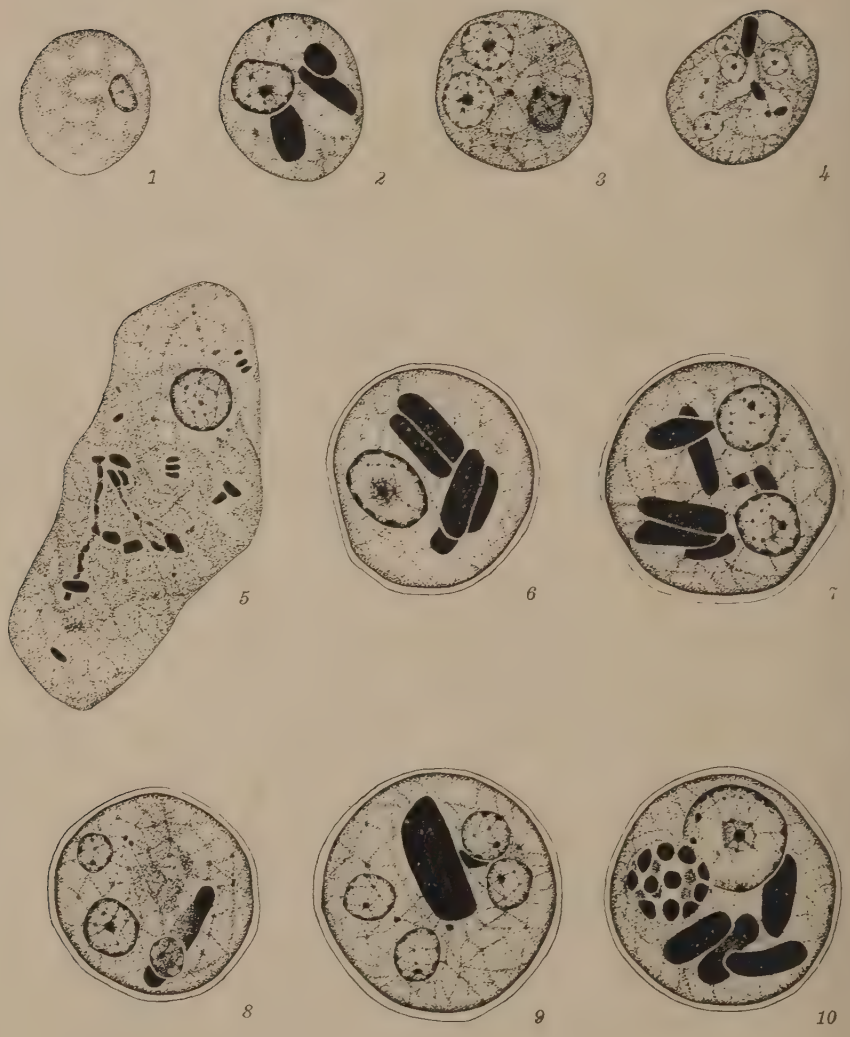

PLATE I

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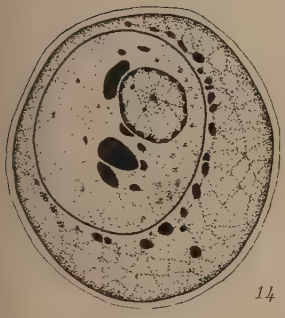
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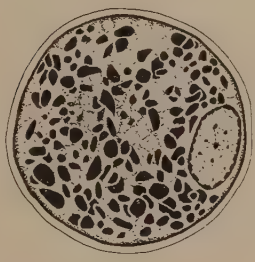
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PLATE II

CERCARIA DOUGLASI CORT, 1917 AND ITS RELATION TO THE CERCARIA OF *COTYLURUS FLABELLIFORMIS* (FAUST, 1917)*

LOUIS OLIVIER AND W. W. CORT

Cercaria douglasi was described briefly by Cort (1917) from a species of *Physa* from the Douglas Lake Region, Michigan. Later Cort and Brooks (1928) identified as this species cercariae from *Lymnaea stagnalis appressa* Say, *L. stagnalis perampla* Walker, *Stagnicola emarginata angulata* (Sowerby), and *Physa parkeri* Currier. Their description, however, was from the cercariae that emerged from three specimens of the varieties of *L. stagnalis*.

Subsequent investigations have revealed that cercariae in the Douglas Lake Region, previously identified as *C. douglasi*, actually represent two distinct but closely related species. One of these, which is found only in members of the PHYSIDAE, we identify as *C. douglasi* Cort, 1917; the other, which develops only in members of the LYMNAEIDAE, was mistakenly identified by Cort and Brooks as *C. douglasi*. This second species has been shown by van Haitsma (1930) to be the cercaria of *Cotylurus flabelliformis* (Faust, 1917).

Since Cort's original description of *C. douglasi* was very brief and was made from cercariae obtained by autopsy from a single snail, a redescription of this species is presented. Also, *C. douglasi* is compared with the cercaria of *C. flabelliformis* with which it has been confused.

REDESCRIPTION

Cercaria douglasi Cort, 1917

(Figs. 1–5)

Specific diagnosis: Strigeid cercaria. (Measurements in Table 1). Oral opening terminal, prepharynx short, pharynx small, esophagus bifurcating about half way between pharynx and level of ventral sucker. Ceca long and narrow, inconspicuous, terminating at level of genital primordium anterior to excretory bladder. Four small, inconspicuous penetration glands anterior to ventral sucker and ventral to ceca. Narrow gland ducts run anteriorly, penetrate oral sucker, in which they are only slightly dilated, and open terminally lateral to oral opening. Excretory system typical. Two pairs of flame cells on each anterior collecting tubule: three pairs on each posterior collecting tubule, two pairs in body and one pair near base of tail stem. A transverse commissure immediately anterior to level of ventral sucker connects anterior portions of bladder arms. A band of 6–8 irregular rows of fine, closely set spines about anterior portion of oral sucker. Ventrally, scattered spines as far back as the anterior margin of genital primordium in lateral fields

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* Contribution from the University of Michigan Biological Station, the Biological Laboratory, New York University, University Heights, and the Department of Helminthology, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.

and as far as ventral sucker centrally. Dorsally, scattered spines just beyond level of pharynx centrally and slightly farther back in lateral fields. About 16–20 long, thin "forward projecting" spines dorsal to oral opening (Fig. 3). Ventral sucker bears several irregular rows of thin, elongate spines. A pair of hair-like processes laterally at level of genital primordium. Hair-like processes on tail stem. Inconspicuous stellate cells grouped along excretory tubule of tail stem. "Island of Cort" small. A pair of clear areas ("unpigmented eyespots") laterally anterior to ventral sucker. Second intermediate hosts are members of Physidae in which tetracotyle larvae very similar to those of *C. flabelliformis* are formed.

Hosts: Physa parkeri Currier from Douglas Lake, Cheboygan County, Michigan. *Physa gyrina* Say from Grace Harbor, Presque Isle County and Munro Lake, Cheboygan County, Michigan.

Like the cercaria of *Cotylurus flabelliformis, C. douglasi* emerges from the snail host during the daytime. The cercariae swim about constantly with tail foremost and frequently change direction. Since they tend to revolve on the long axis as they swim, they often take an irregular spiral course. They tend to be evenly distributed throughout the water in a small container and differ in this respect from the cercariae of *C. flabelliformis* since the latter tend to collect near the surface. This description of the activity of the cercaria of *C. flabelliformis* differs from that given by Cort and Brooks (1928, pp. 191–192); it seems possible that their description was made from old cercariae.

Cercaria douglasi appears to be smaller in all dimensions than the cercaria of *C. flabelliformis* (Table 1). The most striking and clear-cut size difference is in the length of the tail stem, which in *C. douglasi* is always shorter than the body while in the cercaria of *C. flabelliformis* it is always longer. This difference makes it possible to differentiate the two species readily even under low magnification.

TABLE 1.—*Measurements of the cercaria of* Cotylurus flabelliformis *and* Cercaria douglasi. *Both lots of cercariae were fixed in hot 5% formalin by the same method and measurements of 50 of each species made by the same person. All measurements are in microns. The mean with its probable error is given and the extremes are included in parenthesis.*

	Cercaria of *Cotylurus flabelliformis* from the varieties of *Lymnaea stagnalis*	*Cercaria douglasi* from *Physa gyrina*
Body length	217 ± 3 (185–250)	198 ± 2 (172–224)
Body width	39 ± 0.5 (32–51)	38 ± 0.3 (32–45)
Tail stem length	238 ± 1 (211–256)	184 ± 0.5 (173–192)
Tail stem width	36 ± 0.4 (32–45)	32 ± 0.3 (31–38)
Furcal length	239 ± 1 (211–262)	207 ± 1 (186–218)
Length of oral sucker	40 ± 0.3 (32–47)	35 ± 0.4 (29–43)
Width of oral sucker	20 ± 0.1 (18–23)	18 ± 0.1 (13–20)
Diameter of ventral sucker	24 ± 0.1 (21–26)	23 ± 0.1 (21–26)

The two species may also be differentiated on the basis of body spination (Figs. 4 and 5). On the dorsal surface the pattern of spination is virtually the same in both species, but ventrally the spines on the cercaria of *C. flabelliformis* extend to the posterior limits of the body, while on *C. douglasi* they extend only to the level of the genital primordium. In addition, the spines on the cercaria of *C. flabelliformis* are larger and more conspicuous than those of *C. douglasi* so that while the posterior limits of the spination of the latter were difficult to determine, it was relatively easy to determine the extent of spination on the former.

Finally, there appear to be clear-cut differences between *C. douglasi* and the cercaria of *C. flabelliformis* in their choice of second as well as first intermediate hosts, the metacercariae in each case normally developing in snails of the same family as the snails used as first intermediate hosts, i.e., PHYSIDAE for *C. douglasi* and LYMNAEIDAE for *Cotylurus flabelliformis*.

SUMMARY

A redescription of *Cercaria douglasi* Cort, 1917, is presented. This species has been confused with the cercaria of *Cotylurus flabelliformis* (Faust) (= *C. douglasi* Cort and Brooks, 1928). The cercariae of these two strigeid species may be distinguished readily by differences in body spination and relative lengths of their tail stems. *Cercaria douglasi* develops only in members of the PHYSIDAE and members of this family are also the normal second intermediate hosts. The cercaria of *C. flabelliformis* develops only in lymnaeid snails and certain of the LYMNAEIDAE are its normal second intermediate hosts.

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EXPLANATION OF PLATE, p. 346

FIG. 1. Sketch of *C. douglasi* drawn from a living specimen under slight cover glass pressure to show proportions.

FIG. 2. Body and portion of tail of *C. douglasi*. Ventral aspect.

FIG. 3. En face view of *C. douglasi* showing oral opening, gland duct openings and forward projecting spines.

FIG. 4. Body of *C. douglasi*. Ventral aspect. The spined area is indicated by shading.

FIG. 5. Body of the cercaria of *Cotylurus flabelliformis*. Ventral aspect. The spined area is indicated by shading.

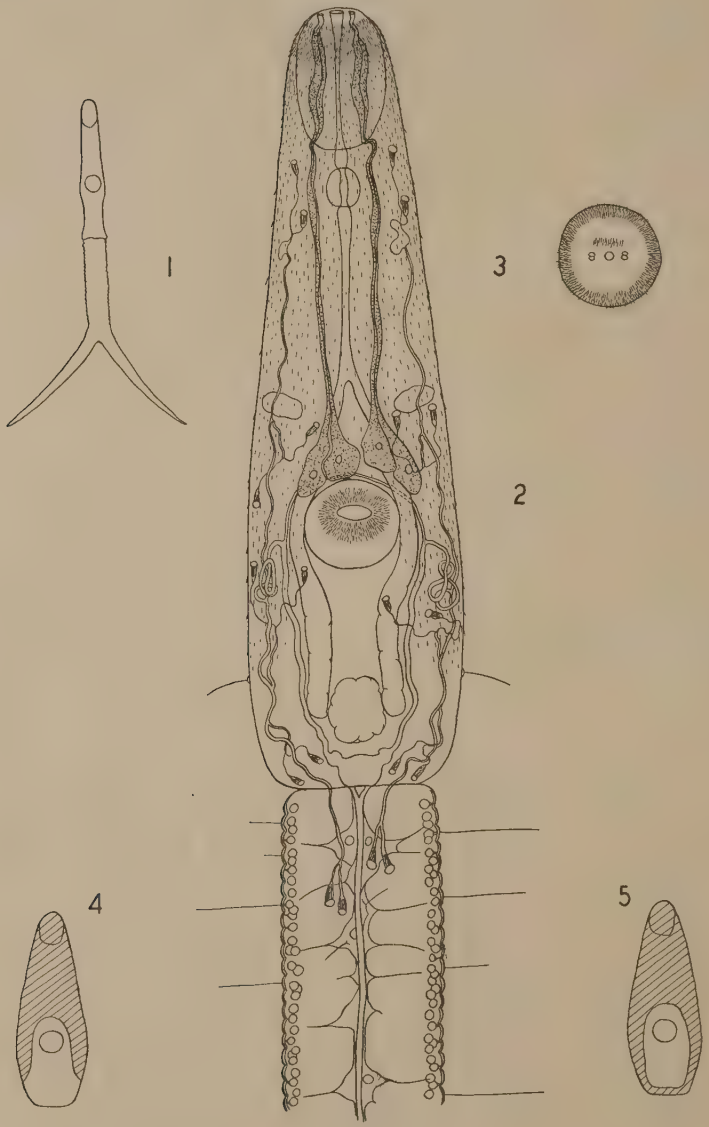

PLATE I

THE LIFE HISTORY OF *ECHINOCHASMUS DONALDSONI* N. SP., A TREMATODE (ECHINOSTOMIDAE) FROM THE PIED-BILLED GREBE*

PAUL C. BEAVER
Lawrence College

In August, 1939, about one-half per cent of *Amnicola limosa* and *A. lustrica* taken from Hook Point on Douglas Lake and from a pond in Wilderness Park, Michigan, were infected with a minute undescribed species of echinostome-like cercaria. The first collections were made by Mr. Alan Donaldson who was surveying cercarial infections in the small operculate snails, and who kindly gave me the echinostome material. Additional material was obtained by numerous subsequent collections. After the cercaria and redia were described, it was possible to undertake a limited number of experiments in an attempt to discover other segments of the life cycle. It was found that freshwater fish are second intermediate hosts and the adult is an undescribed species of *Echinochasmus* which matures in the duodenum of pigeons (experimental) and the pied-billed grebe (*Podilymbus podiceps*). The name *Echinochasmus donaldsoni* is proposed for the new species.

THE CERCARIA

(Fig. 7)

The cercariae emerge in the early morning and are active for 36 to 48 hours in the laboratory. They are extremely small. Swimming behavior is somewhat like that of many stylet cercariae. They swim echinostome-like upwards for a few seconds, then fall slowly with the body flexed ventrally and with the tail contracted and held upward over the ventral side of the body. Creeping is infrequent. Hanging from the surface film is common in water less than a centimeter deep. The body of falling cercariae is circular in outline when seen from above. The tail is long and slender when swimming but may be contracted to less than one-half the length of the extended body when creeping.

Description: Body spindle-shaped except when extended, widest at middle; length 70 to 120 μ in various states of contraction. Tail length about equal that of body. Oral sucker slightly ventral, spherical; diameter slightly greater than that of acetabulum. Acetabulum in posterior third of body, bearing 26 small scale-like

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spines on its margin. Oral sucker bears 10 minute spines on inner dorsal margin and 5 on either side of the outer ventral margin. Collar and collar spines not discernible. Cuticular spines of body undeveloped. Prepharynx relatively long; pharynx cylindrical to spindle-shaped, not pyriform; length less than one-half diameter of oral sucker. Esophagus and ceca plain with open lumina; usually obscure. Cystogenous glands composed of angular masses of fine rods, mostly confined to a lateral field on either side extending from level of pharynx to posterior end, and a median pair of fields between pharynx and acetabulum. Excretory bladder of 2 chambers, both contractile. Excretory siphons arise antero-laterally from anterior chamber of bladder, enlarge immediately and form a single loop between acetabulum and pharynx; siphons narrow rather abruptly at level of pharynx and extend forward to sides of oral sucker. Smaller tubules and flame cells could not be seen. Siphon granules angular and tend to be compound; greatest diameter of largest ones about 4 μ; 8 to 14 in each siphon, evenly distributed along large part of siphons from excretory bladder to pharynx. Tail powerful; attached slightly ventrad. Sharp circumferential ridges throughout its length is a conspicuous feature in contracted tail of living specimens. Excretory canal extending from bladder into tail forms a large top-shaped bladder which may be emptied by contraction; lateral branches of this caudal excretory structure apparently are absent. Pigment absent from all parts of cercaria. Measurements in microns on fixed specimens: body in moderate state of extension, 100 by 52; tail, 102 by 26; oral sucker, 22 by 20; acetabulum, 21 by 18; pharynx, 10 by 9; distance between suckers, 50; distance between oral sucker and pharynx, 15; contracted specimens, body and tail respectively, 70 by 60 and 50 by 30.

THE REDIA

(Figs. 8–10)

Description: Living rediae lightly colored with scattered granules of brownish yellow pigment. Collar plain; low but generally visible on both young and old stages. Appendages usually low, sometimes prominent. Birthpore prominent. Pharynx relatively large, its position variable with state of contraction; sometimes withdrawn far into body, bringing collar forward to anterior tip of body where it may form lips of the oral orifice. Gut usually narrow, long, reaching region of appendages in mature rediae and beyond appendages in very young ones. Cercariae in various stages of development in rediae; no mature cercariae were found in rediae and an abundance of larger immature cercariae were observed free in the snails' tissues. Around 3,000 mature and slightly immature cercariae were found in the tissues of one snail. Measurements on living rediae: largest redia, 1.55 by 0.215 mm just back of collar; sucker, 86 by 77 μ; appendages, 0.34 mm from posterior end. Small rediae, 0.14 mm in length; pharynx, 30 μ wide by 24 μ long. After fixation, collar usually not readily seen; located half-way between birthpore and anterior end. Appendages low; located on posterior one-fifth of body. Gut length as in living state; position ventral, mostly in contact with ventral body wall. Average mature redia, 0.42 by 0.12 mm at level of birthpore; pharynx, 40 μ long by 44 μ wide. Appendages 80 μ from posterior end; collar and birthpore, 50 and 100 μ respectively from anterior end.

THE METACERCARIA

(Fig. 6)

Various species of snails, tadpoles, and fish were exposed to cercariae. All experiments with the first 2 were negative, but every attempt to infect fish was successful.

Small fish and active cercariae were placed in shallow aquaria for several hours. Under magnification it could be seen that the respira-

tory currents were carrying the cercariae into the mouth and pharynx, and the cercariae were but rarely reappearing outside the gill chamber. Examination of such test animals invariably revealed small metacercarial cysts on the gills. Parasite free guppies (*Lebistes reticulatus*), perch (*Perca flavescens*), mollies (*Mollienisia latipinia*), and bluegills (*Helioperca incisor*) were infected in this manner. Additional species which were not definitely known to be parasite free but by exposure and later comparison with control animals from the same lots, were found to be suitable hosts are: mudminnow (*Umbra limi*), bullhead (*Ameiurus nebulosus*), and shiners (*Notropis* spp.). In one of the areas where infected snails were most abundant, minnows were examined for metacercariae which were similar to those found in the experimental minnows. The most abundant species were the five-spined stickleback (*Eucalia inconstans*), fine-scaled dace (*Pfrille neogaeus*), and bowfin (*Amia calva*). Thirty stickle-backs, ranging between 18 and 42 mm in length, carried from 1 to 140 cysts each, with the number of cysts roughly proportional to the size of the fish. Five bowfins ranging between 40 and 50 mm, each carried from 46 to 200 cysts. Twelve dace, around 15 mm in length, carried from 3 to 24 cysts, and 7 larger ones (50 mm) carried from 21 to 170 cysts. In the field hosts the metacercariae were usually in various stages of development.

The mode of infection, encystment, and the nature of development were observed on fish that were infected in the laboratory. Cercariae are carried passively into the gill chamber where they attach themselves to the gill filaments and penetrate the epithelium over or between the lamellae. Occasionally it appears that they establish themselves between the secondary lamellae of smaller fish. In any case, whether their location is deep within the gill tissues or more superficial, they form a relatively strong primary cyst wall immediately. Cysts that are 24 hours old are fairly uniform in size and shape, 68 to 76 μ long by 40 to 44 μ wide, by 34 to 36 μ thick. Where cysts are formed under pressure, the shape may vary slightly. The primary cyst wall is less than 2 μ thick.

The body of the metacercaria does not differ appreciably from that of the cercaria during the first day after encystment. Collar spines become faintly visible after 3 to 6 days and the cyst wall becomes rough and indefinite on its outer margin due to infiltration cells. At 13 days the body is flexed ventrally, collar spines are small but more clearly defined, the cuticula is beginning to show spination pattern, and the cyst wall including host tissue is 4 to 7 μ thick. At 21 days the body is flexed almost double, collar spines are about 3 μ long, cuticular spination is well developed, and the spines around the acetabulum have disappeared. At 4 weeks the spination is slightly advanced and at 5 months the only additional change is in the cyst wall which is 10 to 14 μ thick and unpig-

mented, and the collar spines which are 5 to 7 μ long. Accurate measurements were not obtained on both suckers of a single individual but it appeared that the acetabulum was relatively larger after 4 weeks. Size and shape of the cyst wall, number and distribution of siphon granules, and spination of oral sucker remain unchanged after encystment.

THE ADULT

(Figs. 1–5)

Metacercariae of various ages were fed to 6 pigeons, 6 canaries, 1 chicken, 1 kitten, 1 rat, and 2 mice. Perch that were taken from a lake where snails do not occur were experimentally infected and after 13 days were fed to 3 canaries and 1 pigeon. All were negative after 8 to 10 days. After 17 to 21 days, perch carrying around 150 metacercariae were fed to a pigeon, and 7 days later 3 worms that were barely mature were recovered from the duodeum. Five-spined stickle-backs were collected from an area where the cercariae were found and the approximate number of metacercariae on their gills was determined. They were then fed to a number of hosts to determine whether they would develop into the same adult as those obtained from the experimental metacercariae. Also it was desirable to determine whether metacercariae, some of which probably would be older than 21 days in the field hosts, would develop in laboratory hosts other than the pigeon. Three canaries were given gills bearing 100 to 300 metacercariae, and were found negative after 6, 7, and 9 days respectively. A rat, 2 mice, and a kitten were likewise fed 100 to 300 cysts and were negative on the 8th day. A month-old chicken was negative on the 8th day after receiving 150 cysts from the sticklebacks. Three pigeons were given 100 to 250 cysts and after 7 and 8 days, 1 and 3 mature worms respectively were recovered from the duodenum of 2 of them. Another pigeon was given over a period of 4 days, 250 stickle-backs, each of which carried 1 to 140 metacercariae. Eggs appeared in the feces on the 7th day following the original feeding, and on the 9th day 31 of the worms were found in the duodenum.

Several conclusions may be drawn from the above described feeding experiments. The metacercariae are infective after 3 weeks of encystment. The pigeon is a suitable laboratory host although the percentage of metacercariae which developed into adults was small, possibly because many of the metacercariae were immature. Maturity in the adult worm is reached on the 7th day in pigeons. Its location in the host is the duodenum. The canary is definitely refractive to this species and although the experiments with the rat, mouse, kitten, and chicken were inconclusive, it seems likely that they too are unsuitable laboratory hosts. The adults obtained from the field infections of metacercariae are the same as those from the experimental infections.

Two pied-billed grebes (*Podilymbus podiceps*) were taken from an area about 25 miles from where the infected snails were collected. In the first one, 25 worms which were identical with those taken from experimental pigeons, were found in the duodenum. Over 100 worms of this species were taken from the second.

The following description is based on sections and toto-preparations of mature specimens from the grebe and pigeon.

Description: Mature worms small and elongate, tapered at neck and posterior end. Size, 0.88 by 0.256 mm to 1.84 by 0.32 mm. Widest level usually at, or just posterior to, testes but may be at level of acetabulum, esophagus, or crown. Cuticular spines relatively large, extending to level of posterior testis along lateral and ventral sides, and to ovary on dorsal side. Oral sucker small, terminal, with the mouth directed ventro-anteriorly; provided with a row of 10 scale-like spines on the inner border of the dorsal side and 8 to 10 low papilla-like elevations of the cuticula over the ventral and lateral surfaces of the outside border. Pharynx oval; length equal to, or greater than diameter of oral sucker. Walls of esophagus somewhat irregular, narrow anteriorly but usually much inflated near bifurcation. Ceca compressed laterally throughout most of length and extend to posterior end where they are attached to, or at least in contact with, the terminal chamber of the excretory bladder. Acetabulum spherical, near middle of body; diameter about twice that of oral sucker. Crown wedge-shaped, bearing 20 relatively large spines; arranged 9 on either side in a single row that is interrupted dorsally, and 1 on each angle. Angle spines usually much smaller than others but are sometimes almost equal to them; margin spines nearly uniform in size and shape.

Testes contiguous, round to elongate transversely; anterior testis typically wider and usually larger than posterior one; margins simple. Ovary on right side; diameter varies from slightly less than, to 1.5 times the length of ova. Oviduct arises dorsally and enlarges to form a distinct fertilization chamber just proximal to junction of Laurer's canal; Laurer's canal long, containing sperms in inner portion; outer portion very narrow and apparently without opening to outside; attachment to body wall directly dorsal to left intestinal cecum. Yolk reservoir voluminous, receiving right and left vitelline ducts separately. Yolk ducts pass ventral to ceca in reaching vitellaria. Vitelline follicles extend from level of acetabulum to posterior end, the lateral fields joined back of testes by 5 or 6 isthmuses ventral to the excretory bladder and 3 or 4 dorsal to it. Uterus short, forming 3 to 6 horizontal and vertical loops; sperms stored in posterior portion of uterus. True seminal receptacle absent. Metraterm on left side and somewhat ventral to cirrus sac. Genital atrium shallow, simple. Cirrus sac ovoid, usually widest at its anterior end; mostly dorsal to acetabulum, usually extending far beyond middle of acetabulum and sometimes reaching its posterior border. Seminal vesicle voluminous, divided into posterior and anterior chambers. Pars prostatica and prostate gland small but distinctly present. Cirrus extremely short and simple. Genital pore crowded between acetabulum and gut bifurcation. Excretory bladder divided into a longitudinal series of 5 chambers; terminal chamber lined with irregular cuboidal epithelium which is continuous with the intestinal ceca although apparently there is no communicating lumen between the two structures. Lateral excretory siphons large in preacetabular region. Measurements on average specimen: length, 1.51 mm; width at level of crown, pharynx, acetabulum, and anterior testis respectively, 0.248, 0.170, 0.256, and 0.240 mm; oral sucker, 70 by 65 μ; pharynx, 80 by 60 μ; diameter of acetabulum, 153 μ; cirrus sac, 160 by 84 μ; diameter of ovary, 70 μ; eggs, 72 by 54 to 76 by 50 μ; anterior testis, 120 by 154 μ; posterior testis, 118 by 146 μ; spines on margin of crown, 66 by 14 to 72 by 15 μ; angle spines of crown, 40 by 10 μ; length of cuticular spines at level of genital pore, 10 μ.

Host: Podilymbus podiceps.
Habitat: Duodenum.
Locality: Indian River, Michigan, U. S. A.
Paratype specimens: U. S. Nat. Mus. Helm. Coll. Nos. 36723–36725.

DISCUSSION

The cercaria of *Echinochasmus donaldsoni* is closely related to *Cercaria ornatostoma* Cable, 1935. The behavior and general shape of the body, suckers, and tail are similar; cuticular and collar spines are lacking in both; cystogenous glands consist of angular masses of small slender rods; and the excretory bladder, ducts, and granules are identical in pattern. Oral spines occur on the ventral and dorsal lips of the oral sucker, and similar spines form a single row on the margin of the acetabulum. The rediae of the two species are likewise similar in the character of the collar, appendages, birthpore, and sucker; the gut is long in both. *C. ornatostoma* is much the larger of the two species, moderately extended specimens measuring 0.2 mm in length while specimens of the cercaria of *E. donaldsoni* are only 0.12 mm when fully extended. The oral sucker of *C. ornatostoma* bears a ring of about 12 large perioral cells that are not found in the other species, and the oral spines of the ventral lip are 8 in *C. ornatostoma* instead of 10. Siphon granules are preacetabular in *C. ornatostoma* while they extend into the postacetabular region in the other. *C. ornatostoma* was found in *Goniobasis semicarinata* in Kentucky.

There are 27 more or less well known species which have been placed in the genus *Echinochasmus* Dietz, 1909. In 1931, Price reviewed the genus and listed the known species. Since then the following species have been added: *E. novalichensis* by Tubangui (1932); *E. rugosus* and *E. rediduplicatus* by Yamaguti (1933); *E. ruficollis* by Ishii (1935); *E. ruficapensis* and *E. baqulai* by Verma (1935); *E. narayani* by Mudaliar (1938); *E. gorsakii*, *E. milvi*, and *E. tobi* by Yamaguti (1939); and *E. reniovarus* and *E. megavitellus* by Lal (1939). Because the vitellaria in *E. reniovarus* extend anteriorly into the preacetabular region, it must be removed from the genus *Echinochasmus* and placed in the genus *Episthmium* Lühe, 1909. It therefore becomes *Episthmium reniovarus* (Lal, 1939).

The numerous species of the genus *Echinochasmus* form 4 sub-groups, based on the number of collar spines. Four species, all Japanese, have 28 or more collar spines; 15 species have 24; 6 species have 22; and *E. dietzevi* Issaitschikoff, 1927, like the form described here, has but 20 collar spines.

Comparison of *E. dietzevi* with the 20-spined North American form reveals a number of striking differences if the description of the former may be regarded as accurate for typical normal specimens. *E. dietzevi* has 3 alternating angle spines while *E. donaldsoni* has 1 only. Back of the

testes, the vitellaria form rather solid fields that almost meet at the mid-ventral line and are confluent dorsally in *E. dietzevi.* In *E. donaldsoni* the vitellaria of the two sides are joined by isthmuses along both the dorsal and ventral sides. The intestinal ceca extend almost to the body wall at the posterior end in *E. dietzevi,* leaving no space for an inflated portion of the terminal part of the excretory bladder such as is found in *E. donaldsoni.* Except for the differences in spine pattern, the other differences might easily be explained as due to relatively poor specimens, or lack of detail in description. There is perhaps still some question as to whether distinct species of echinostomes may have identical spine pattern, but there cannot be the least doubt that individual collections of worms with different collar spine patterns are distinct species.

Although the life cycle of *E. perfoliatus* (Ratz) is somewhat imperfectly known (Muto, 1921), apparently it is the only species of the genus whose development, modes of transmission, and hosts are known to closely resemble those of *E. donaldsoni.* The cercariae of both are minute gymnocephalous forms which differ principally in the distribution of the siphon granules and pigmentation of the cystogenous gland cells. In both species the cercariae are carried into the pharynx of freshwater fish where they encyst on the gill filaments, and when infective metacercariae are eaten by the final host, maturity in the adult stage is reached in a relatively short period, 7 to 10 days. The metacercariae of *E. japonica* Tanabe (Ujiie, 1936) and *E. liliputanus* (Looss) (Ciurea, 1931) also are found on the gills of freshwater fish.

SUMMARY

1. A new gymnocephalous cercaria is described from *Amnicola limosa* and *A. lustrica* from the region of Douglas Lake, Michigan, and its life cycle demonstrated experimentally.

2. The cercariae encyst on the gills of numerous species of small freshwater fish and develop collar spines typical of echinostomes.

3. Metacercariae are infective after 3 weeks and become adults in the duodenum of pigeons, maturing on the 7th day.

4. The pied-billed grebe (*Podilymbus podiceps*) is a field host for the adult.

5. The adult is described as a new species, *Echinochasmus donaldsoni.*

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EXPLANATION OF PLATE

Drawings made with the aid of a camera lucida.

FIG. 1. Young adult. Ventral view.

FIG. 2. Young adult. Lateral view.

FIG. 3. Head crown of adult. Ventral view.

FIG. 4. Head crown of adult. Lateral view.

FIG. 5. Composite transverse section of mature worm at level of ovary, fertilization chamber, Laurer's canal, ootype, etc. Anterior view.

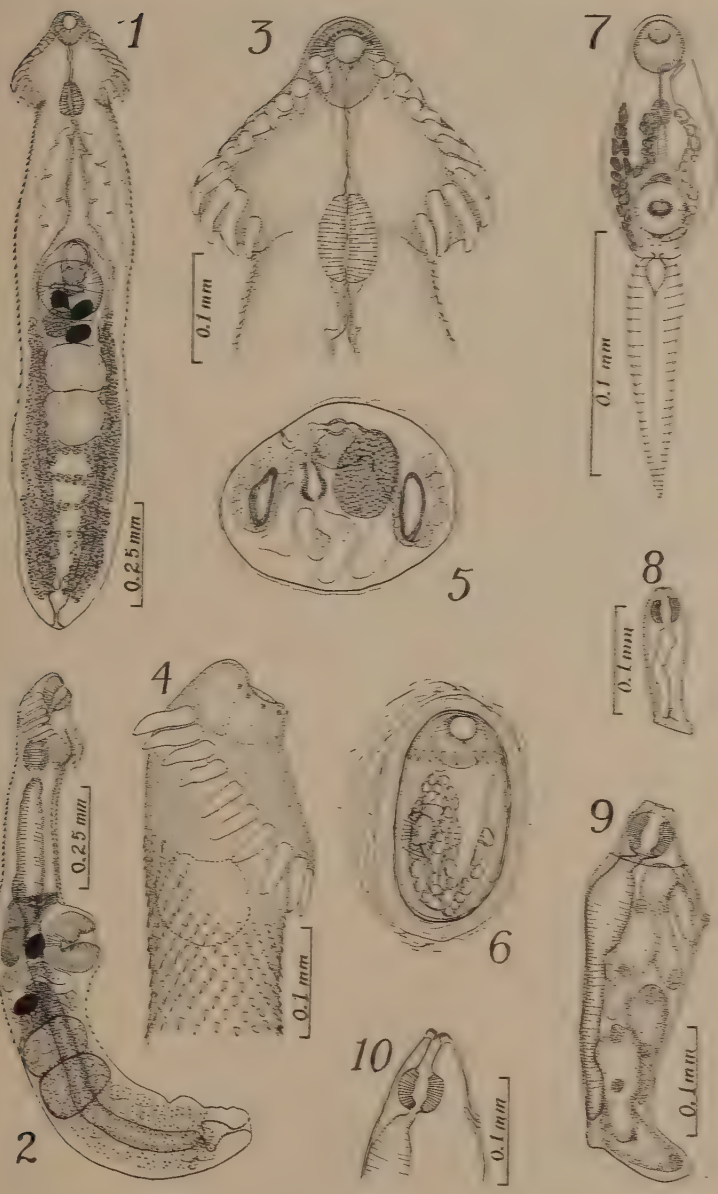
FIG. 6. Metacercaria, 4 weeks after encystment.

FIG. 7. Cercaria. Ventral view, moderately contracted. (Outlines drawn from fixed specimen.)

FIG. 8. Immature redia. Ventro-lateral view.

FIG. 9. Mature redia. Lateral view, moderately contracted.

FIG. 10. Anterior end of mature redia showing pharynx withdrawn to level of birthpore and the collar constricting the opening at the extreme anterior tip.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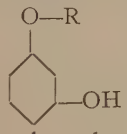
ANTHELMINTIC STUDIES ON THE MONO ETHERS OF THE DIHYDROXY BENZENES

J. W. Landsberg and C. F. Lischer
Warner Institute for Therapeutic Research, New York City

The anthelmintic activity of the resorcinols and their derivatives has been studied extensively in recent years, particularly by Lamson and his associates. As is well known the most desirable member of this class of compounds, viewed from the standpoint of both anthelmintic activity and toxicity, is hexylresorcinol. There is another group of closely related compounds, however, the mono ethers of the dihydroxy benzenes, having the general formula  whose anthelmintic activity has not been investigated as far as the authors are aware.

Some of the members of this class of compounds occur naturally (guaiacol, beechwood creosote, oil of clove), while others (resorcinol, hydroquinone, catechol) must be prepared synthetically. Although it is recognized that many more compounds of this series exist, only the compounds having a normal arrangement of carbon atoms from C_4 to C_6 were prepared since this range has been demonstrated by other investigators to be the most effective as anthelmintics. The organic synthesis of these members was carried out by one of us (C. F. L.) and will not be considered here in detail. It is sufficient to say that the compounds had the proper structure and the degree of purity required by the accepted standards.

EXPERIMENTAL METHODS

The anthelmintic activity of the compounds studied was determined by the in vitro method of Lamson and Brown, the mechanics of which have been previously described (Landsberg and Accousti (1)). The test object, *Ascaris lumbricoides* of swine, was obtained at a local slaughter house. The worms were exposed to the drug and then tested for motility, by dropping into water at 60° C, twenty-four hours after exposure. If no motion in either direction was observed they were considered dead.

To determine the toxicity of the compounds studied the usual pharmacological procedures were carried out. As a preliminary measure graduated doses, based on grams per kilo body weight, were given to adult rats by stomach tube. By this method the dose which would kill in twenty-

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four hours was determined. This dose then was administered to each of 10 rats and the mortality in twenty-four hours determined. Since the objective was to determine the dose which would produce a fifty per cent mortality (L. D. 50) in twenty-four hours, this dosage was raised or lowered until that quantity of drug was found. In some instances this involved several groups of 10 rats each. When the fifty per cent killing dose was established a final 10 animals were used for confirmation.

EXPERIMENTAL RESULTS

As a preliminary experiment runs were made on the various mono ethers using two ascarids for each run. The concentration used in each instance was one to a thousand. The results have been summarized in Table 1.

TABLE 1.—*Preliminary runs on the mono ethers of the dihydroxy-benzenes*

Compound*	Time								
	2′	5′	10′	20′	30′	45′	1 hr	2 hr	3 hr
	Percentage of worms killed								
Resorcinol mono-n butyl ether ..	0	50	100	100	100	100	100	100	100
Resorcinol mono-n amyl ether ..	0	100	100	100	100	100	100	100	100
Resorcinol mono-n hexyl ether ..	0	0	100	100	100	100	100	100	100
Hydroquinone mono-n butyl ether	0	0	66	100	100	100	100	100	100
Hydroquinone mono-n amyl ether	0	0	33	100	100	100	100	100	100
Hydroquinone mono-n hexyl ether	0	0	50	100	100	100	100	100	100
Catechol mono-n butyl ether ...	0	0	0	0	0	0	0	100	100
Catechol mono-n amyl ether ...	0	0	0	0	0	0	50	100	100
Catechol mono-n hexyl ether ...	0	0	0	0	0	50	100	100	100
Guaiacol	0	0	0	0	0	50	100	100	100
Beechwood Creosote	0	0	0	0	50	50	100	100	100
Iso Eugenol	0	0	0	0	0	0	100	100	100
Eugenol	0	0	0	0	0	0	100	100	100
Oil of Clove	0	0	0	0	0	50	50	100	100

* Concentration 1 : 1,000.

From this table it may be observed that resorcinol mono-n butyl ether, resorcinol mono-n amyl ether and resorcinol mono-n hexyl ether had the best anthelmintic activity. The first killed fifty per cent in 5 minutes, the second killed one hundred per cent in 5 minutes and the third killed one hundred per cent in ten minutes.

Since only two worms were used in the preliminary runs, other runs were made on the three compounds showing the most promise, using larger numbers of ascarids. In each instance 12 worms were removed at each time interval, and as all the worms were killed after twenty minutes of exposure, the experiment was terminated after one-half hour.

From Table 2 it may be observed that resorcinol mono-n hexyl ether gave the best results, killing fifty-five per cent of the ascarids in 2 minutes and one hundred per cent in 5 minutes. At this time it should be pointed out how the additional number of worms shifted the killing power of the

TABLE 2.—*Comparison of some of the mono ethers of dihydroxy-benzene with hexylresorcinol*

Compound*	Time in minutes				
	2	5	10	20	30
	Percentage of worms killed				
Resorcinol mono-n butyl ether	0	10	73	100	100
Resorcinol mono-n amyl ether	40	42	73	100	100
Resorcinol mono-n hexyl ether	55	100	100	100	100
Hexylresorcinol	75	100	100	100	100

* Concentration 1 : 1,000.

compound. When only two worms were run, resorcinol mono-n amyl ether appeared to have the most activity, killing one hundred per cent in 5 minutes (Table 1), and resorcinol mono-n hexyl ether appeared to have less activity than any of these three compounds, not killing one hundred per cent until ten minutes (Table 1). When 12 worms were removed at each time interval, however, using the same concentration, the killing ability of the compounds was shifted markedly. In this instance resorcinol mono-n hexyl ether showed the most activity, killing one hundred per cent in five minutes (Table 2) while resorcinol mono-n amyl ether did not kill one hundred per cent until 20 minutes exposure (Table 2).

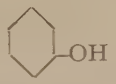
It is recognized that hexylresorcinol has probably the best anthelmintic activity of this general class of compounds. With this in mind the three compounds prepared by us were compared with hexylresorcinol by the same in vitro method. The results of this comparison have been summarized in Table 2. It may be observed from this table that the anthelmintic activity of resorcinol mono-n hexyl ether is closer to that of hexylresorcinol than any of the other compounds of this series. On the basis of 12 worms removed at each stated time interval resorcinol mono-n hexyl ether killed fifty-five per cent after two minutes exposure as compared with seventy-five per cent killed by hexylresorcinol for the same period under the same experimental conditions. At the five minute period, however, the two compounds had equal killing power, both killing one hundred per cent.

Since the in vitro anthelmintic activity of resorcinol mono-n hexyl ether and hexylresorcinol is very close, varying only twenty per cent at two minutes exposure (Table 2), it appeared desirable to determine the relative toxicity of these two compounds. One of the common pharmacological procedures, used for the determination of an acute dose, is the establishment of the L. D. 50 for twenty-four hours, i.e., the dosage which will kill fifty per cent of the experimental animals in twenty-four hours.

From a preliminary study on rats it was found that 1.75 gm resorcinol mono-n hexyl ether per kilo of rat, killed in twenty-four hours. This dosage, after further testing in other rats, was found to be the L. D. 50 for twenty-four hours. When the same dosage of hexylresorcinol (1.75 gm per kilo) was given to rats the killing power was essentially the same. On the basis of toxicity in rats then, resorcinol mono-n hexyl ether does not appear to be more toxic than hexylresorcinol.

DISCUSSION

On the basis of the experimental procedures outlined previously, resorcinol mono-n hexyl ether has the best anthelmintic activity of the group of fourteen mono ethers of the dihydroxy benzenes studied. In passing it may be observed also that of these compounds the synthetic members have greater anthelmintic activity than those occurring in nature (Table 1). By the same in vitro testing method resorcinol mono-n hexyl ether appears to have about the same anthelmintic activity as hexylresorcinol. The toxicity of this compound in the rat on a grams per kilo basis compares favorably with hexylresorcinol.

Resorcinol mono-n hexyl ether is a reddish-brown, oily liquid having the formula 

. Its solubility is close to that of hexylresorcinol. Lamson et al. (2) in describing the physical properties of a phenol required for good ascaricidal activity, maintain that it should be a liquid and have a solubility ranging from 1 : 1,000 to 1 : 35,000. It may be observed that resorcinol mono-n hexyl ether falls into such a category. It is realized fully that the activity observed in the in vitro ascaris testing procedure does not give an absolute index of anthelmintic activity in either man or other animals, but in view of the observations it appears to the writers that this compound, resorcinol mono-n hexyl ether, merits additional investigation. Further studies should include the in vivo action on the dog ascaris and the toxicity of the compound for canines with the resultant pathology. Upon the basis of these results the efficacy of the compound for other animal parasites, as well as for human administration, may be considered.

SUMMARY

1. Of the fourteen compounds of the mono ethers of dihydroxy benzene, resorcinol mono-n hexyl ether appears to have the greatest anthelmintic activity when tested by the in vitro ascaris technique.

2. The toxicity of this compound, when tested on rats, is no greater than that of hexylresorcinol.

3. In the opinion of the authors this compound merits further investigation, particularly in vivo anthelmintic and toxicity studies in dogs.

Bibliography

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2. Lamson, P. D., Brown, H. W., Stoughton, R. W., Harwood, P. D., Baltzly, R. and Bass, A. D. 1935 J. Pharmacol. and Exper. Therap. **53**: 239–249.

RESEARCH NOTES

OCCURRENCE OF *TRICHOSTRONGYLUS COLUBRIFORMIS* IN THE GUINEA PIG

A male specimen of *Trichostrongylus colubriformis* 4.5 mm long was discovered in the posterior part of the small intestine when a young guinea pig, experimentally infected with *Trichinella spiralis*, was examined for adult trichinae. Autopsy of another young guinea pig, purchased at the same time and used in the same experiment, did not reveal any trichostrongylids, nor could helminth eggs be demonstrated in a fecal sample from its rectum.

Altogether, of a large number of trichinized guinea pigs whose intestines have been examined for adult trichinae, this was the only case in which accidental infection with another worm was met with. The guinea pig in question had been purchased from a man who bought up laboratory animals from small landowners in the vicinity of Copenhagen. The animal must be supposed to have picked up the infective larva when it was fed with fresh grass from an area where infected sheep were running. Then, the very young age (few months) of the guinea pig may have been one of the causes why such an unusual parasite could reach maturity in its intestine.

T. colubriformis, a very common parasite of the sheep, has also been reported from a long series of other hosts, as goat, cattle and other ruminants, horse, squirrel, hare, rabbit, zizel, several kinds of apes and monkeys, and man.—HANS ROTH, *Institute for General Pathology, University of Copenhagen (Director: Professor Oluf Thomsen)*.

NOTE ON LIFE HISTORIES OF THE GENUS *PARAMONOSTOMUM* LÜHE, 1909 (TREMATODA: NOTOCOTYLIDAE) WITH SPECIAL REFERENCE TO THE EXCRETORY VESICLE

During the past five years repeated efforts have been made to solve the life-histories of the notocotylid cercariae parasitizing *Planorbis ulvae* (Pennant, 1777). The experiments have been tried again this winter with partial success.

Six species of these larvae were isolated and the cysts fed to laboratory reared ducklings. Three pertained to the Monostomi sub-group and three to the Yenchingensis sub-group. Although the material of the former group was far more plentiful all efforts at rearing the adult worms again failed. Two species of the Yenchingensis sub-group, however, developed in the intestinal ceca of the ducks, into flukes of the genus *Paramonostomum*.

It is well known that in mature notocotylid trematodes the excretory vesicle becomes greatly complicated. The essential change lies in the development of numerous large ramifying branches arising from the lateral edges of the vesicle. This system is not visible in preserved material and has only been figured by Looss (1896, Mem. Inst. Egypt. **3**: 1–252) for *Catatropis verrucosa* (Frölich, 1789) and by Noble (1933, Tr. Am. Micr. Soc. **52**: 353–359) for *C. pacifera* Noble, 1933. In these drawings there is no structure shown which could represent the unpaired diverticula present in Yenchingensis cercariae. In this region (the extreme anterior portion of the vesicle) there are a pair of branches figured, one lying on either side of the esophagus.

The excretory system of the adult *Paramonostomum* is very similar to that of *Catatropis*: large branches developing along the lateral edges of the vesicle. These are difficult to trace to their extremities, but in some very favorable specimens it appears that the branches arising from the inner margin anastamose in the center line of the body, thus forming a network. The median unpaired diverticula of the cercaria persists as a large conspicuous unpaired branch immediately overlying the esophagus (Figs. 1 & 2).

It will be recalled that in an earlier publication (Rothschild, 1938, Novit. Zool. Tring, **41**: 75–83) the author divided the notocotylid cercariae into three groups

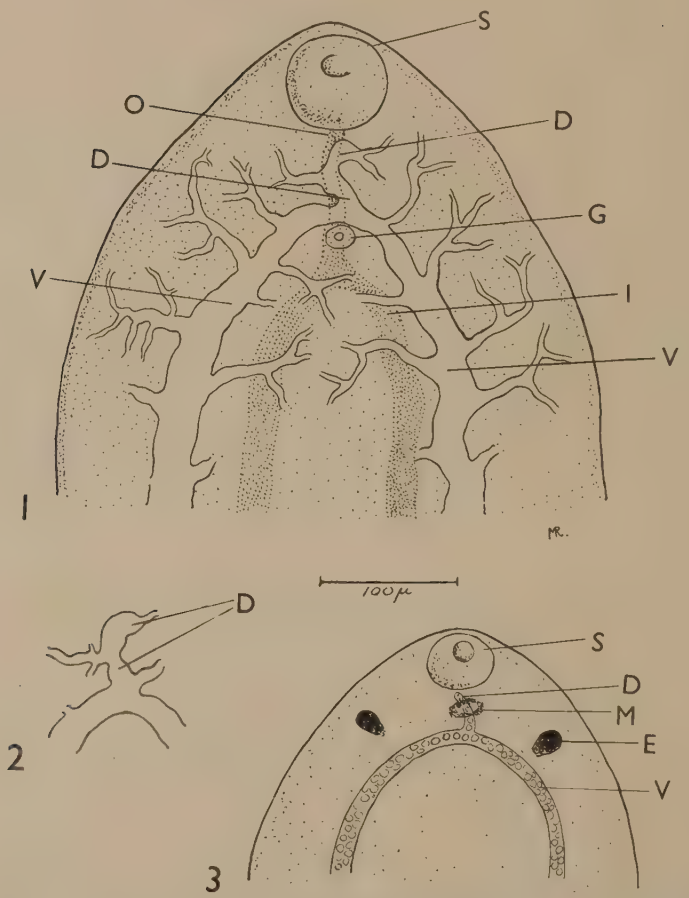

Fig. 1. Anterior end of *Paramonostomum* sp. showing the excretory vesicle. The diagram was drawn with the aid of a camera lucida during life and the finer branches are omitted. Anastamosing of the median branch could not be made out in this specimen.

Fig. 2. The unpaired diverticula in another specimen.

Fig. 3. Anterior end of the cercaria of *Paramonostomum* sp. (Yenchingensis sub-group) showing the unpaired diverticula of the excretory vesicle. (Camera lucida drawing.)

Abbreviations

D—unpaired diverticula of excretory vesicle
E—lateral eye spot
G—genital pore
I—intestine
M—median eye spot
O—esophagus
S—oral sucker
V—excretory vesicle

according to the structure of the anterior portion of the excretory vesicle. (It is probable that a fourth group may be now added in which the two halves of the excretory vesicle do not fuse in the anterior region, but remain separate, as in the earlier stages of cercarial development. This is suggested by Herber's interesting experiments (Herber, 1939, J. Parasitol. **25**: Suppl: 18–19.) and some unpublished drawings shown to the author by Prof. R. P. Dollfus. This character will probably be found to characterize the adult worm as well as the larva.) It is therefore of interest to find that the unpaired diverticula of the cercaria (Fig. 3) persists in the adult *Paramonostomum*. However, the genus *Notocotylus* as it now stands may well contain two or more types of excretory vesicle. Thus at least one cercaria of the Yenchingensis group and one of the Monostomi group (Szidat, L. and U., 1933, Centr. Bakt. I Abt. Orig. **129**: 411–122; Yamaguti, 1938, Z. Parasitenk. **10**: 288–292) have been reported to develop into the same species of adult, *Notocotylus attenuatus* (Rud. 1809)!

Further investigation into the excretory system of adults of the Monostomi subgroup is therefore very desirable and renewed efforts are being made to rear those species found in *Peringia ulvae*.—Miriam Rothschild, *Peterborough, England.*

THERMAL DEATHPOINT OF THE ERYTHROCYTIC STAGES OF *PLASMODIUM CIRCUMFLEXUM*

Very little work has been done on the thermal deathpoint of the malaria parasite. In their paper on cultivation Bass and Johns (1912, J. Exper. Med. **16**: 567) stated that glass pipettes recently flamed should be allowed to cool below 50° C since otherwise the blood and parasites might be injured. The present study was undertaken since we needed to know the thermal deathpoint of *P. circumflexum* in the erythrocytic cycle in connection with immunity experiments.

Two strains of *P. circumflexum* were used. In earlier work (Manwell and Goldstein, 1940, J. Exper. Med. **71**: 409) these were designated as Strains E and A, and a history of the strains was presented. Both strains are virulent and show exo-erythrocytic stages with regularity.

The technique used to determine the deathpoint was of two sorts. At first the parasites were placed in a constant temperature water bath. But it was found that results obtained this way did not differ markedly from those made by placing the blood in a constant temperature incubator equipped with a De Khotinsky thermal control. Since this latter method was simpler, it was used in most of the experiments. The vial containing the blood and parasites was then placed in a vessel filled with water at the same temperature as the incubator. This step was to prevent any changes of temperature which might be produced by opening and closing the incubator door.

The blood was removed with sterile precautions from a heavily infected canary and mixed with either citrated physiological saline, or saline to which heparin had been added. This was then placed in a glass vial and sealed air-tight with a sterile piece of rubber dam, after taking pains to remove all of the sulphur bloom from the rubber. The vial was now put into the hot water bath or into the incubator. In all experiments, two minutes were given for the glass vial and fluid to reach the experimental temperature. Every five minutes the vials were shaken.

A total of 47 female canaries was used. Of this number, 36 were experimental while 11 served as controls. 33 were Strain E cases; 3 Strain A. All injections of previously heated material were intravenous.

The results are summarized in Table 1.

It is clear from these data that the thermal deathpoint of erythrocytic stages of *P. circumflexum* is 50° C for 15 minutes of exposure; or at 55° C for 5 minutes of heating. This assumes, of course, that sufficient time has been allowed for the warming of the vial and fluid to the experimental temperature. It would be difficult to establish the exact point of death and a more complicated technique would have to be employed to this end. But for general purposes, the above range is sufficiently accurate. At 40° C there was no effect of temperature on the parasites even when ex-

posed 30 minutes. At 45° C there was a delayed prepatent period over that of the controls of from 3 to 6 days in samples exposed longer than 15 minutes. This is indicative that some of the parasites were killed by the heating while others resisted it, or that the parasites were weakened but recovered. At 50° C any exposure over 15 minutes resulted in no infection, while 5 and 10 minute exposures caused a delay similar to that observed at 45° C for 15 minutes. At 55° C five minutes of exposure was sufficient to kill all of the parasites.

TABLE 1.—*Effect of temperature on prepatent period and parasites*

Exposure time (minutes)	40°C. (1 case each)	45°C. (2 cases each)	50°C. (3 cases each)	55°C.
5	No effect	No effect	Delayed	No infection (12 cases)*
10	No effect	No effect	Delayed	No infection (3 cases)
15	No effect	Delayed	No infection	No infection (5 cases)
30	No effect	Delayed		

* 9 cases of Strain E and 3 cases of Strain A.

An interesting fact is that blood heated to 55° C for even 15 minutes shows little morphological change when smeared and stained with Giemsa. Naturally the color had darkened much during exposure to the heat. The parasites in such blood show very little change in shape other than a tendency to round up. The main apparent change in the parasites occurs in the staining since they appear darker in color, both the chromatin and cytoplasm. But merozoites look very much like normal ones except, again, that they stain more darkly.

Conclusions: The thermal deathpoint of *P. circumflexum* in red blood cells appears to be 50° C when exposure is for 15 minutes, or 55° C for 5 minutes approximately. There is no hemolysis of the blood at these temperatures. The parasites undergo little morphological change other than a tendency to round up and take a deeper stain with Giemsa.—FREDERICK COULSTON, *Department of Zoology, Syracuse University.*

BALANTIDIUM COLI AND PINWORM IN A CHIMPANZEE

In 1935 a young chimpanzee in the Chicago Zoological Park at Brookfield, Illinois, was reported by its attendant to be ailing. A fecal sample was sent to the writer. The sample contained considerable mucus but was formed. Microscopic examination revealed a moderate *Balantidium coli* infection.

Five days later another fresh sample was obtained. It was fluid with much mucus. One active female pinworm was found, as well as many eggs. The number of balantidia had greatly increased over that in the previous sample. Both cysts and trophozoites were plentiful.

The above is a record of a double infection, helminthic and protozoan. The correlation between the diarrheic condition of the fecal sample and the increase in the number of balantidia is marked. Hegner (1934, Am. J. Hyg. 19: 480–501) states that in fecal examination of fifteen chimpanzees the impression was gained that the animals most heavily infected with balantidia had the greatest tendency to become diarrheic. The presence of the pinworm may have been a factor contributing to the diarrhea in the case of the Brookfield chimpanzee.—ALBERT E. MOORMAN, *Kansas Wesleyan University, Salina, Kansas.*

REVIEW

CARLOS FINLAY AND YELLOW FEVER. By Carlos E. Finlay. xii + 249 pp., 25 illustrations. Oxford University Press, New York. 1940.

No short review can do justice to this attractive volume, which brings together biographical notes on Carlos Finlay, the story of the development of his theory of mosquito transmission of yellow fever, the record of the results obtained in the control of yellow fever following the confirmation of his theory in 1900, and a discussion of recent laboratory and epidemiological findings in the light of his experimental work.

The purpose of the volume is best stated in the author's own words:

> "The misconception of my father's role in the discovery of the transmission of yellow fever by the mosquito, its undervaluation and the nonrecognition of the experimental phase of the work, which has prevailed in almost all the discussions of yellow fever in the English language, constitute the principal inducements which have led me to write this book."

This labor of filial love has been carefully executed and the result is a most excellent brief, brought up to date on the basis of laboratory and field work during 1927 and 1940, for the plaintiff in the case of *Finlay vs Reed et al, and Gorgas:* by a coincidence it has the same number of pages as has the Senate Document No. 822 (1911), which may be considered as the brief for the defense. The two volumes should be studied together.

It is indeed regrettable that unpleasant discussions as to who should get the credit for the mosquito transmission of yellow fever should have occurred; surely there was glory and to spare for all who had any part in contributing to the work which led to the control of the most fearful of tropical scourges.

Such discussions were, however, unavoidable since Finlay believed so utterly in the doctrine of mosquito transmission that ". . . it is a question if his faith in it was increased by the results of Reed's experiments" (Carter), but was unable to convince others; ". . . in the trying period from 1881 to 1900 only two figures stand forth in the background: one was my mother, his wife and faithful helpmate . . .: the other was Dr. Claudio Delgado, at that time my father's only disciple. . . ." In spite of exceptional opportunities to present his theories before scientific societies in Cuba, the United States and Europe, and personally to such students of the disease as Sternberg and Gorgas, Finlay never succeeded in convincing others of the validity of his experimental work. Reed and Carroll, who became part of the Army Commission in Cuba in 1900, had been working on the etiology of yellow fever since 1897 and turned to the theory of mosquito transmission only after all other leads had been played out and after Carter's field observations indicated the necessity for an "extrinsic incubation period" between the first case and secondary cases in any community.

It is perhaps natural that advantage should have been taken of these discussions by strong nationalists and others with political axes to grind:

> ". . . those flocking after him because of his theory did so from patriotic and other motives which before had not appealed to them" (page 25).

One cannot but consider as suspect such actions as that cited on page 44:

> "Finally, at the closing session of the German Ibero-American Academy held at Vienna in August, 1939, a resolution was unanimously carried recognizing *Carlos J. Finlay as the sole discoverer of the mosquito transmission of yellow fever.*"

It is hardly to be expected that the historians of the future will accept this sweeping conclusion with its implications of criminal negligence of health officers who permitted the uncontrolled ravages of yellow fever from 1881 to 1901.

Surely, it will not be difficult to reconcile rival claims in the face of (1) Reed's declaration, page 219:

> "We here desire to express our sincere thanks to Dr. Finlay who accorded us a most courteous interview and has gladly placed at our disposal his several publications relating to yellow fever, during the past nineteen years; and also for ova of the species of the mosquito with which he has made his several inoculations . . . with the mosquitoes thus obtained we have been able to conduct our experiments."

and (2) Finlay's statements in 1903:

> "*I have never claimed that I had demonstrated the factors, but I have proved by unmistakable evidence, in print, that I had foretold the results which the Army Board afterwards confirmed, giving them as original discoveries of their own.*" (The transmission of yellow fever. J. Am. Med. Assn., 1903, p. 1660).

It is to be expected that the final decision will be in accord with the tribute paid by Doctor Sawyer on his reception of the "Carlos J. Finlay" decoration May 16, 1940, which forms the final paragraph of the Appendix of the book under review:

> "The decoration which I am receiving at your hands might appropriately be considered a recognition of the yellow fever research of my colleagues as well as my own, for we always work as a group, and I am especially pleased to learn that one of them, Dr. Fred L. Soper, is being similarly honored on this occasion. The decoration is especially dear to me, as a student of yellow fever, because it bears the name and likeness of Carlos Finlay, who made the first great contribution to our knowledge of the disease. Far ahead of his time, and at the very beginnings of bacteriology, he published a hypothesis that yellow fever was transmitted by a special mosquito and strove for two decades to prove his thesis, experimentally to the satisfaction of his contemporaries. The fact was finally firmly established for him, and with his friendly collaboration, by the Army Commission. During the long period in which his thesis was refused acceptance and was even scorned, he held firmly to his convictions and at last he was able to see the full acceptance of his theory and its application to the immense benefit of man. In accepting this decoration I pay homage to Carlos Finlay."

Attention should be called to the fact that the Bibliography as given fails to cite the following publications:

1888 The microbe of yellow fever. Medical Record, New York, **33**: 622.
1889 Sur le Micrococcus versatilis (Sternberg) C. Delgado and C. Finlay J. Anat. et Physiol. **25**: 223.
1896 Apéndice á la comunicación sobre el tetracoca versatilis y la fiebre amarilla. 8 pp. 8°, Habana.
1899 Work cited by Reed. Medical Record, New York, **55**(21). May 27, 1899.
1900 Etiologia de la infección hemogastrica en la fiebre amarilla. Rev. Med. y Cir., Habana, **5**: 117–122. Also 1899, An. Acad. **36**: 183–190.

—Fred L. Soper, *Rio de Janeiro, Brazil.*